INSTRUCTION MANUAL

MODEL L-7250 PROGRAMMABLE AUTOSAMPLER

BASIC OPERATION

Read and keep this manual

- Read safety instructions carefully and understand them before starting your operation.
- Keep this manual at hand for reference.

HITACHI

INSTRUCTION MANUAL

FOR

MODEL L-7250 PROGRAMMABLE AUTOSAMPLER

BASIC OPERATION

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INSTRUCTION MANUAL FOR MODEL L-7250 PROGRAMMABLE AUTOSAMPLER

PREFACE

The L-7250 Programmable Autosampler is designed to enable analysis of multiple samples when you operate it as part of a liquid chromatography system.
This instruction manual has been prepared for users of Model L-7250 programmable autosampler. It describes the operation, checkup and maintenance procedures for the L-7250 programmable autosampler.
The Model L-7250 is intended for use by persons having a basic knowledge of chemical analysis. Remember that improper use of the analytical instruments, chemicals or samples would result not only in wrong analytical data but also in consequences adverse to safety.
Read this instruction manual carefully before attempting operation. For proper use of this instrument, please acquaint yourself with it.

The instruction manual for the L-7250 Programmable Autosampler consists of two volumes: BASIC OPERATION and ADVANCED OPERATION. The volume "BASIC OPERATION" contains the fundamental procedures and introductory explanations of technical matters. The volume "ADVANCED OPERATION" includes higher-level procedures, such as programming.
Please read through the volume "BASIC OPERATION" before using the instrument and attempting any of the more advanced operations.

IMPORTANT

Warranty on Product

(1) Scope of Warranty

The Model L-7250 programmable autosampler is warranted to be free from defects in material, workmanship and operation under normal use within the product specifications indicated in this manual for the period stated below. Liability under this warranty is limited to repair, adjustment or replacement by Hitachi or its approved representative.
Any parts which prove to be defective during the warranty period will be repaired, adjusted or replaced without charge. Note that a substitute part may be used for repair, or replacement with an equivalent product may be made instead of repair. Also, such system components as a personal computer and printer to be updated frequently for improvement may not be available in original versions at the time of replacement.
The warranty contained herein is for the benefit of and shall be enforceable by the original purchaser of this instrument and is not transferable.
Consumable parts and operating supplies are excluded from this warranty.

(2) Warranty Period

One year from the date of initial installation.
(In case a separate warranty document has been issued, the warranty period indicated in it takes precedence over the above period.)

(3) Limitations and Exclusions on Warranty

Note that this warranty is void in the following cases:

(a) Failure due to operation at a place not meeting the installation requirements specified by Hitachi.

(b) Failure due to power supply voltage/frequency other than specified by Hitachi or due to power failure.

(c) Corrosion or deterioration of the tubing due to impurities contained in reagent, gas or cooling water supplied by the user.

(d) Corrosion of the electric circuits or deterioration of the optical elements due to highly corrosive atmospheric gas.

(e) Failure due to use of hardware, software or spare parts other than specified by Hitachi.

(f) Failure due to improper handling or maintenance by user.

(g) Failure due to maintenance or repair by a service agent not approved or authorized by Hitachi.

(h) Failure due to relocation or transport after initial installation.

(i) Failure due to disassembly, modification or relocation not approved by Hitachi.

(j) Failure due to acts of God, including fire, earthquake, storm, flood, lightning, social disturbance, riot, crime, insurrection, war (declared or undeclared), radioactive pollution, contamination with harmful substance, etc.

(k) Failure due to computer virus infection.

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NO ORAL OR WRITTEN INFORMATION OR ADVICE GIVEN BY HITACHI, ITS DEALERS, DISTRIBUTORS, AGENTS OR EMPLOYEES SHALL CREATE A WARRANTY OR IN ANY WAY INCREASE THE SCOPE OF THIS WARRANTY.

HITACHI ASSUMES NO LIABILITY FOR ANY DAMAGE TO DATA OR APPLICATION SOFTWARE DUE TO ANY POSSIBLE FAULT OR FAILURE OF THIS INSTRUMENT.

Installation, Relocation and After-Sale Technical Service

Installation of this instrument shall be carried out by or under supervision of qualified service personnel of Hitachi or its authorized service agent.
Before installation of this instrument, the customer shall make preparations for satisfying the installation requirements in accordance with this manual.
When relocation of this instrument becomes necessary after initial installation (delivery), please notify your local Hitachi sales representative or nearest Hitachi service office.
Technical support service for this instrument is available from a service agent approved or authorized by Hitachi within regular working hours on workdays.

Disposal of This Instrument

When disposing of this instrument, follow the relevant environmental protection regulations for your local requirement.

Other Precautions

(1) Handling of Chemicals and Samples

(a) The user is responsible for following relevant legal standards and regulations in the handling, storage and discarding of chemicals and samples used in analytical operations of this instrument.

(b) Reagents, standard solutions and accuracy-control samples shall be handled, stored and discarded as instructed by the respective suppliers.

(2) Notice on This Instruction Manual

(a) The information contained in this manual is subject to change without notice for product improvement.

(b) This manual is copyrighted by Hitachi with all rights reserved. No part of this manual may be reproduced or transmitted in any form or by any means without the express written permission of Hitachi.

⚠ SAFETY SUMMARY

⚠ General Safety Guidelines

Before using the L-7250 programmable autosampler, carefully read the safety instructions given below.

- Operate the instrument according to the instructions in this manual.
- Be sure to observe the warnings indicated on the product and in the instruction manual. Failure to do so could result in personal injury or damage to the product.

- The hazard warnings which appear on the warning labels on the product or in the manual have one of the following alert headings consisting of an alert symbol and a signal word DANGER, WARNING or CAUTION.

⚠	**DANGER** :	Indicates an imminently hazardous situation which, if not avoided, will result in death or serious injury. (This warning does not apply to this product.)
⚠	**WARNING** :	Indicates a potentially hazardous situation which, if not avoided, can result in death or serious injury.
⚠	**CAUTION** :	Indicates a hazardous situation which, if not avoided, will or can result in minor or moderate injury, or serious damage to the product.
⚠	:	The alert symbol shown at left precedes every signal word for hazard warnings, and appears in safety-related descriptions in the manual.
	Important :	Used for inastructions other than the abve in order to prevent damage to the instrument.
	Note :	Used for information and descriptions that are provided to ensure correct usage.

⚠ SAFETY SUMMARY

⚠ General Safety Guidelines (Continued)

- Keep in mind that the hazard warnings in this manual or on the product cannot cover every possible case, as it is impossible to predict and evaluate all circumstances beforehand.
  Be alert and use your common sense.

- When using a chemical for analytical operation, be sure to provide proper ventilation of the laboratory room per local requirements. Inadequate ventilation could endanger human health.

- Do not modify the instrument, replace parts that are not user-serviceable, use non-specified parts, nor remove safety devices, as it could be hazardous.

- Installation at delivery, maintenance and relocation should be referred to service personnel qualified by Hitachi.

- Do not perform any operation or action other than described in this manual. When in doubt, please contact your local Hitachi sales representative or nearest Hitachi service office.

⚠ SAFETY SUMMARY

⚠ WARNING: Ignition of Flammable Chemicals!

Handling of Flammable Chemicals

- Beware of ignition hazard when using flammable chemicals such as organic solvents.
- Always check the following conditions. If an abnormality is found, stop operation immediately.
  - ◇ Leakage of solvent or waste solution
  - ◇ Leakage of solvent inside the instrument
  - ◇ Inadequate ventilation of the laboratory room
- This instrument is not explosion-proof. During unmanned operation, do not use organic solvents having an ignition point below 70°C.
- When using flammable chemicals, be careful about possible ignition due to static electricity. Particularly when using non-conductive chemicals, employ a conductive vessel made of metal or the like and provide grounding connection correctly.

⚠ WARNING: Explosion of Vapor from Flammable Chemicals!

Handling of Flammable Chemicals

- If a flammable chemical such as organic solvent leaks from the flow path of the instrument and its vapor concentration exceeds the explosion limit, it may cause spontaneous combustion with dangerously explosive results.
- When using a flammable and readily volatile chemical, be sure to check for leakage from the instrument flow path and ventilate the laboratory room adequately.

⚠ SAFETY SUMMARY

⚠ WARNING: Electric Shock in Contact with Inside of Instrument!

Beware of Electric Shock.
Potentially Dangerous Voltages are Present within the Instrument.

- Before removing the instrument cover for replacement or adjustment of internal parts, be sure to turn OFF the power switch and unplug the power cord.

⚠ WARNING: Electric Shock due to Improper Grounding!

Ground Properly to Prevent Electric Shock Hazard.

- Be sure to use the power cable supplied with the instrument. Use of a different power cable may result in an electric shock hazard.
- This instrument is classified as "1" in IEC1010-1 Annex H and "plug-connected type" in IEC1010-1, so connect the power cable to a grounded 3-wire outlet.
- If a grounded 3-wire outlet is not available, then be sure to provide proper grounding connection.

⚠ SAFETY SUMMARY

⚠ CAUTION: Explosion of Lithium Battery!

Handling of Lithium Battery

- This instrument uses a lithium battery for memory backup. It will explode if not handled properly.
- Never recharge, disassemble or incinerate the lithium battery. When discarding the lithium battery, treat it separately from other waste.
- When it becomes necessary to replace the lithium battery with a new one, notify your local Hitachi sales representative or nearest service office.
- Refer servicing of lithium battery replacement to qualified service personnel who have received relevant technical training.
  After the warranty period of this instrument, replacement service is available at charge.

⚠ CAUTION: Injury during Operation of Instrument!

Caution during Instrument Operation

- Never touch the inside of autosampler or put a thing into it during operation, as your hand or finger may be injured. Be especially cautious when the arm, needle or syringe is in action.

⚠ SAFETY SUMMARY

⚠ CAUTION: Touching Hot Part may Result in Burns!

Caution on Use of Constant-Temperature Rack for Autosampler

- The constant-temperature rack for autosampler remains hot for a while even after power-off and can severely burn you if touched.
- When handling the constant-temperature rack, be careful not to touch its hot part.

⚠ SAFETY SUMMARY

NOTICES:

Restriction on Use of Reagents or Samples

- Fluorocarbon resin, stainless steel (SUS316), borosilicated glass, Vespel, PEEK, EPDM and polypropylene are used in the flow path of this instrument. Never use reagents that would corrode these materials.
- When measuring a strongly acidic or alkaline sample, be sure to attach the septum and screw cap on the sample vial. Otherwise, the inside of this instrument will corrode to cause a failure. Note that any sample exceeding pH range '1 to 10' is not usable on this instrument.

Precautions on Use of Corrosive Solvents

- The drain path for carrying leakage solutions is made of polypropylene.
- The materials inside the instrument are susceptible to corrosion by strong acid, strong alkali and organic solvents.
- When using corrosive solvents, make sure that the tubing connections are not loose.
- Using the pump pressure limiter function or by other means, make setting so that liquid delivery is stopped automatically if leakage occurs.

Precautions on Disposal of Waste Solution

- Be sure to collect waste solution and treat it properly for disposal. Improper disposal treatment of waste solution may result in environmental pollution.

Precaution on Accuracy/Precision of Measured Values

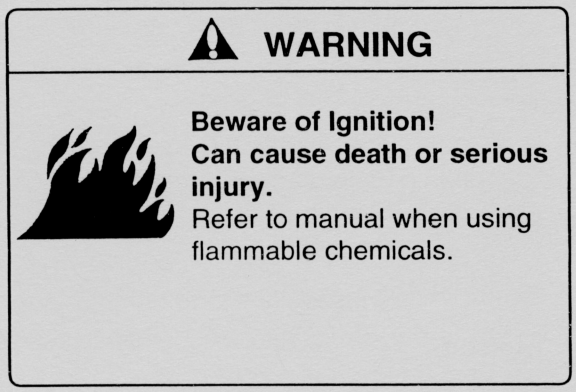
- Use control sample measurements to ensure that the performance of the instrument is normal.

⚠ SAFETY SUMMARY

WARNING LABELS

The warning labels shown below are attached on the Model L-7250 programmable autosampler.

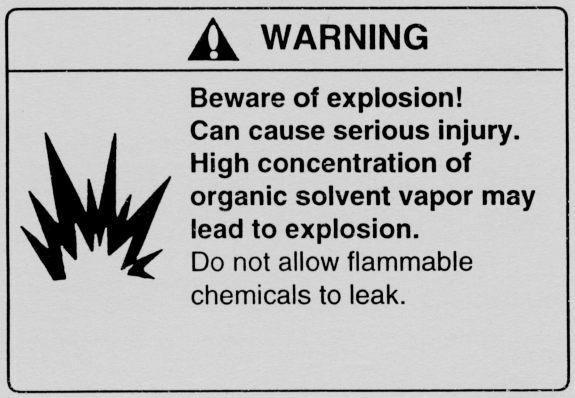
(1) Ignition of Flammable Chemicals



[Attached position]
On right rear side

810-1951

(2) Explosion of Vapor from Flammable Chemicals

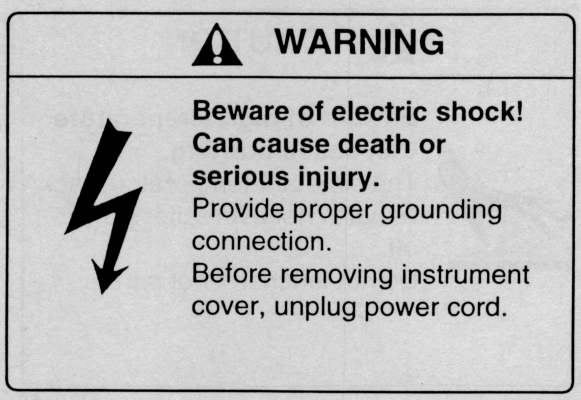


[Attached position]
On right rear side

810-1952

⚠ SAFETY SUMMARY

(3) Electric Shock in Contact with Inside of Instrument



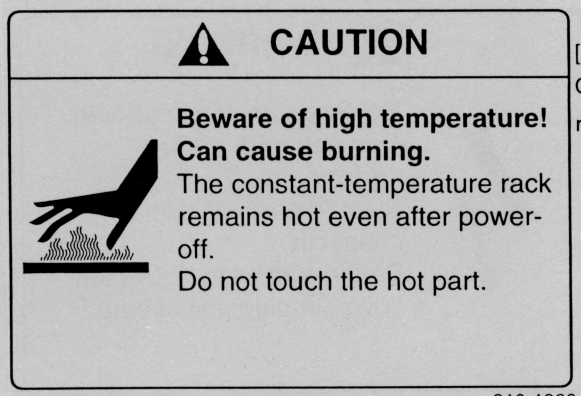
810-1954

[Attached position]

On left rear side

⚠ SAFETY SUMMARY

(4) Burns upon Touching Hot Part



[Attached position]
On constant-temperature rack (option)

810-1960

(5) Injury by Needle

[Attached position]
On right front side

810-1961

Contents

PREFACE 1

IMPORTANT 3

Warranty on Product 3

Installation, Relocation and After-Sale Technical Service 6

Disposal of This Instrument 6

Other Precautions 6

SAFETY SUMMARY SAFETY–1

NOTICES SAFETY–7

WARNING LABELS SAFETY–8

1 PREPARATIONS (BASIC PROCEDURES)

1.1 About the Liquid Chromatography System 1–1

1.2 Overview of the L-7250 Programmable Autosampler 1–2

1.2.1 Overall Design of the Unit 1–2

1.2.2 Flow Path 1–3

1.3 The Instrument Keypad 1–4

1.4 Precautions 1–6

1.5 Routine Operations 1–10

1.6 Setting of Simple Analysis Conditions 1–12

2 BASIC OPERATIONS

2.1 Preparatory Operations 2–1

2.2 Outline of System Operations 2–3

2.3 Operation of the Autosampler 2–5

2.3.1 Routine Operations 2–5

2.3.2 Setting the Analysis Conditions 2–6

2.3.3 Quick Sample Analysis 2–29

2.3.4 Confidence Level Report 2–31

2.4 Stop Procedures 2–34

2.5 Operational Precautions 2–35

2.5.1 Setting the Cut Volume and the Syringe Speed 2–35
2.5.2 Injection Volume and Cut Volume 2–35
2.5.3 Precautions when Performing Injections Using the All Volume Injection Method 2–36
2.5.4 Notes on Sample Injection with the Full Loop Method 2–37
2.5.5 Eluent and Wash Solution 2–37

3 PRINCIPLES, FUNCTIONS AND SPECIFICATIONS

3.1 Application 3–1
3.2 Function 3–1
3.2.1 Configuration 3–1
3.2.2 Functions 3–2
3.2.3 Sample Injection Methods 3–2
3.3 Specifications 3–6

4 INSTALLATION

4.1 Unpacking 4–1
4.2 Checking Contents in Package 4–1
4.3 Installation Requirement 4–1
4.3.1 Electrical Power and Space Requirements 4–1
4.3.2 Environmental Considerations 4–2
4.3.3 Items to be Provided by User 4–4
4.4 Assembly 4–4
4.4.1 Layout 4–4
4.4.2 Assembly 4–5
4.4.3 Tubing Connections 4–8
4.4.4 Wiring 4–10
4.5 Operation Check 4–15
4.6 Performance Check 4–15

5 MAINTENANCE AND PERIODIC CHECKING OF THE SYSTEM

5.1 Periodic Maintenance 5–1
5.1.1 Washing the Flow Path 5–1
5.1.2 Cleaning 5–3
5.1.3 Waste Liquid Tube 5–3

5.2 Checking the Performance and Specifications 5–3
5.2.1 Checking the Reproducibility 5–3
5.3 Positioning the Mechanism 5–10
5.3.1 Adjusting the Height of the Vial Detection Lever 5–10
5.3.2 Adjusting the Needle Position (height) 5–11
5.4 Troubleshooting 5–13
5.4.1 Troubleshooting Table 5–13
5.4.2 On Occurrence of Failure 5–15

6 PARTS REPLACEMENT

6.1 Consumables and Spare Parts 6–1
6.1.1 Consumables 6–1
6.1.2 Spare Parts 6–2
6.2 Injection Port Seal 6–2
6.3 Needle Replacement 6–3
6.4 Syringe Replacement 6–5
6.5 Replacing the Injection Valve Seal 6–6
6.6 Replacing the Syringe Valve Seal 6–7

APPENDIX

A.1 Error Message List A–1
A.2 Connecting Components via the D-line Communication Feature A–3
A.3 Terminology A–8

INDEX I–1
STANDARD OPERATING PROCEDURE (SOP)

1 PREPARATIONS (BASIC PROCEDURES)

This chapter covers the basic operational procedures of the L-7250 Programmable Autosampler. Begin with the basic operation only after you have completed the installation procedures given in Chapter 4.

1.1 About the Liquid Chromatography System

The L-7250 Programmable Autosampler automatically withdraws a sample from a sample vial and injects it into the mobile phase flow path of a liquid chromatograph. The analyst can control the sample volume, the time between injections, and those related parameters that allow for unattended operation.

Figure 1-1 shows an overview of the various components of a liquid chromatography system. This figure includes the autosampler.

To ensure maximum performance of the liquid chromatograph and to reduce the possibility of downtime, check the following points on a routine basis before attempting to operate the unit.

Main Check Points for the System	
(1)	Pump • Ensure that the flow rate setting is correct • Check that you have chosen the appropriate pressure limit • Check that there is sufficient eluent in the eluent bottle
(2)	Autosampler • Check that there is sufficient wash solution in the wash solution bottle • Ensure that there is enough space in the autosampler waste solution bottle to accommodate the anticipated waste
(3)	Column • Ensure that the appropriate column is in place • Check that the maximum pressure is set on the pump module
(4)	Detector • Verify the correct wavelength setting • Ensure that there is enough space in the detector waste bottle to accommodate the eluent from the column
(5)	Integrator • Confirm that there is sufficient chart paper in the unit • Verify that you have the appropriate data processing parameters
(6)	Other Inspections • Check for leaks in the system (tighten joints if necessary) • Check for the presence of dried salts around joints (clean and tighten if necessary) • Ensure that there are no constrictions or obstructing bends in the tubing

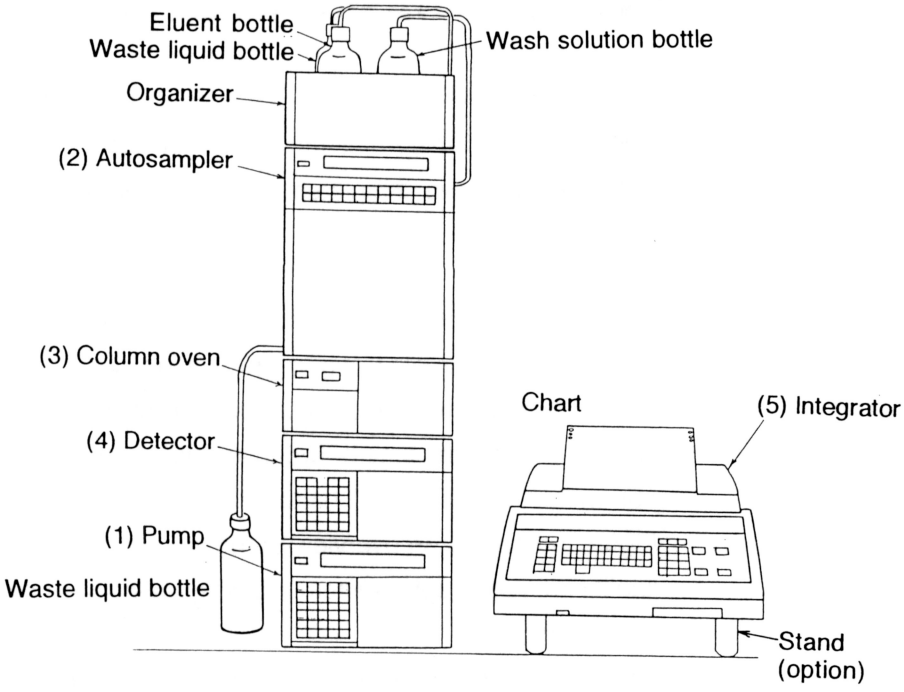


Figure 1-1 Typical Configuration of the Liquid Chromatography System

1.2 Overview of the L-7250 Programmable Autosampler

1.2.1 Overall Design of the Unit

Figure 1-2 is a photograph of the L-7250 Programmable Autosampler. A chamber houses a rack to store the samples, the syringe, and a sampling arm. The sampling arm moves along the transverse axis (X), the longitudinal axis (Y), and the vertical axis (Z) to withdraw the desired sample and place it in the injection valve. The injection valve includes a sampling loop to inject samples into the mobile phase flow.

All operational conditions for the L-7250 Autosampler are set via the keypad on the face of the unit. The display panel presents information about the status of the system.

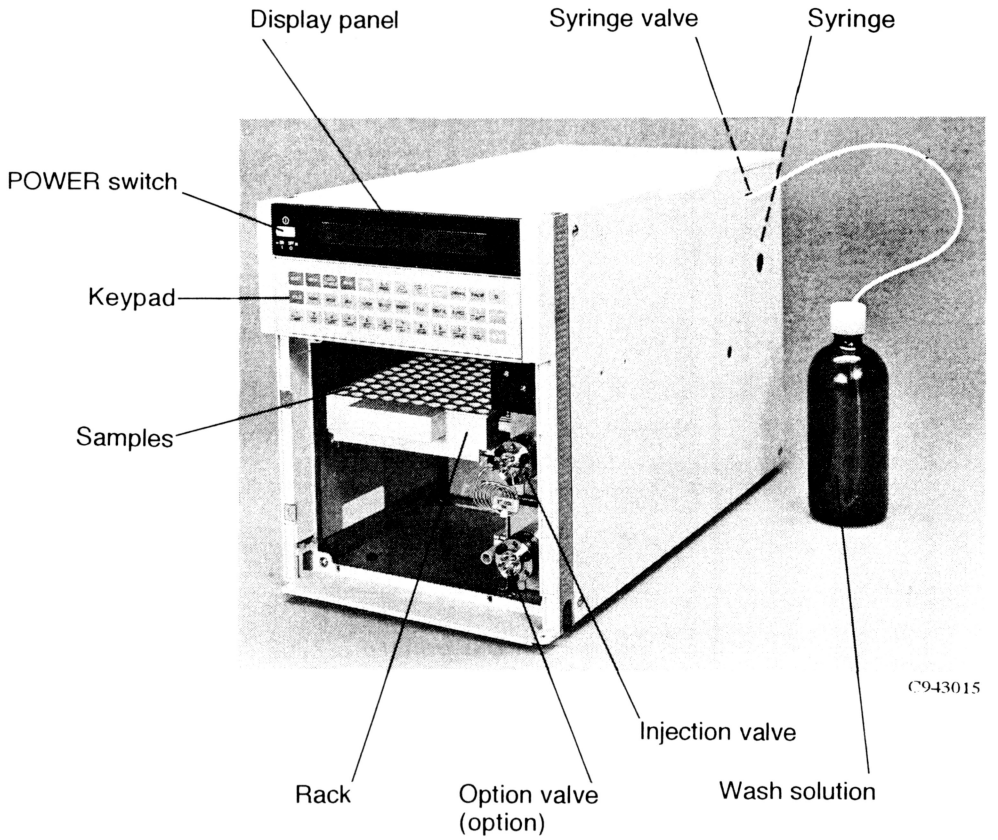


Figure 1-2 Configuration of the L-7250 Programmable Autosampler

1.2.2 Flow Path

Figure 1-3 includes the various components of the system that are in contact with the sample and the wash solution in the L-7250 Autosampler. The needle moves along the X, Y and Z axes to permit free access between sample vials, the wash port, and the injection port. The action of the syringe withdraws sample from the sample vial and delivers it to the injection port.

A cycle includes the following steps:

- Cleaning of the needle at the wash port
- Aspirating of a sample solution by the needle
- Loading of the sample solution into the loop of the injection valve
- Switching of the injection valve to inject the sample into the mobile phase

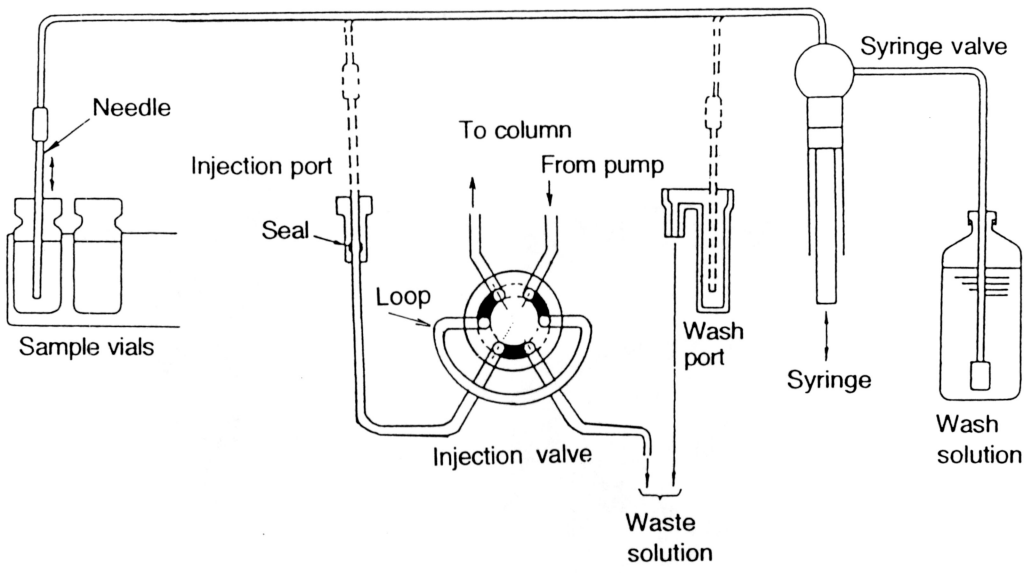


Figure 1-3 Flow Path Diagram of L-7250 Autosampler

1.3 The Instrument Keypad

Figure 1-4 shows the operation keys of the L-7250.

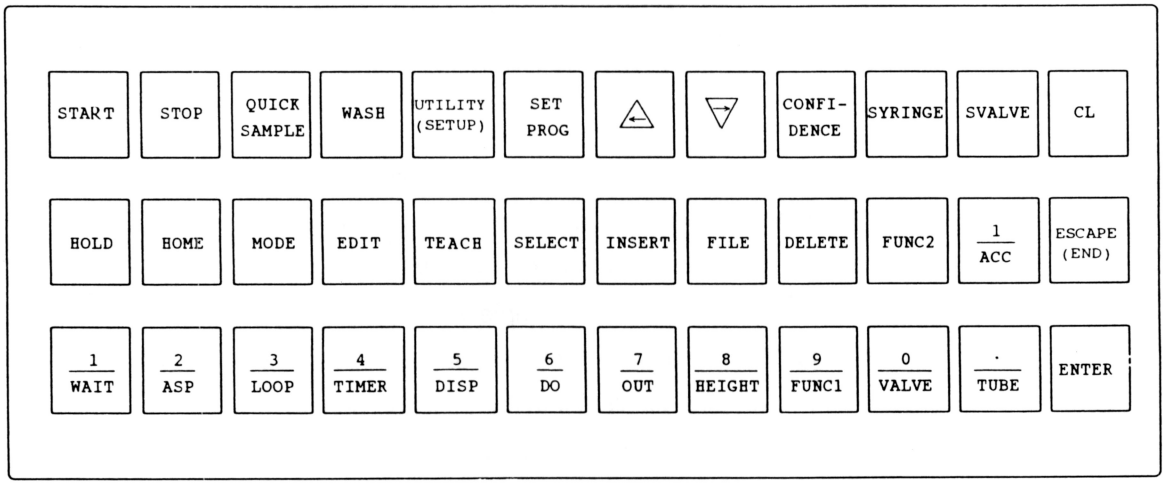


Figure 1-4 The Keypads of L-7250 Autosampler

START	Starts analysis.
STOP	Stops analysis.
HOME	Returns the needle and other moving parts to their original positions. It is used after an interruption.
WASH	Washes the needle and the injection valve's flow paths. Also used for aspirating the wash solution in the flow path of the syringe.
QUICK SAMPLE	Measures nonscheduled samples.
MODE	Selects a sequential or a programming mode.
HOLD	Pressing this key, sets up the wait state to prevent injection of the next sample. Pressing HOLD again, cancels the wait state and allows injection of the next sample.
SET PROG	Sets the sample injection conditions for sequential mode.
UTILITY (SETUP)	Sets the basic operating conditions.
CONFI-DENCE	Provides confidence-level reporting.
◁ ▽	These keys let you scroll up/down the screen. Use these keys for step changeover in the programming mode. In the robotic mode, use them to position the needle.
ESCAPE (END)	Press this key after the basic analysis conditions are set or to interrupt setting of the analysis conditions.
CL	Deletes data while in the process of being defined (prior to pressing ENTER).
ENTER	Enters numerical values.

Programming mode key : Use the programming mode keys to create programs for sample preparation. Refer to the Advanced Operation Instruction Manual for additional information.

Note : Press **ESCAPE (END)** key if in doubt while setting the analysis conditions. Pressing **ESCAPE (END)** will usually displays the initial screen.

1.4 Precautions

1. Waste Bottle and Waste Tube

⚠ WARNING

Ignition of Flammable Chemicals!

- Beware of ignition hazard when using flammable chemicals such as organic solvents.
- For normal flow of waste solution, be sure to follow the instructions given in Figure 1-5.
- Always check the flow path of waste solution. If an abnormality is found, stop operation immediately and then take a proper countermeasure.
- When using flammable chemicals, be careful about possible ignition due to static electricity.
  Particularly when using non-conductive chemicals, employ a conductive vessel and provide grounding connection correctly.

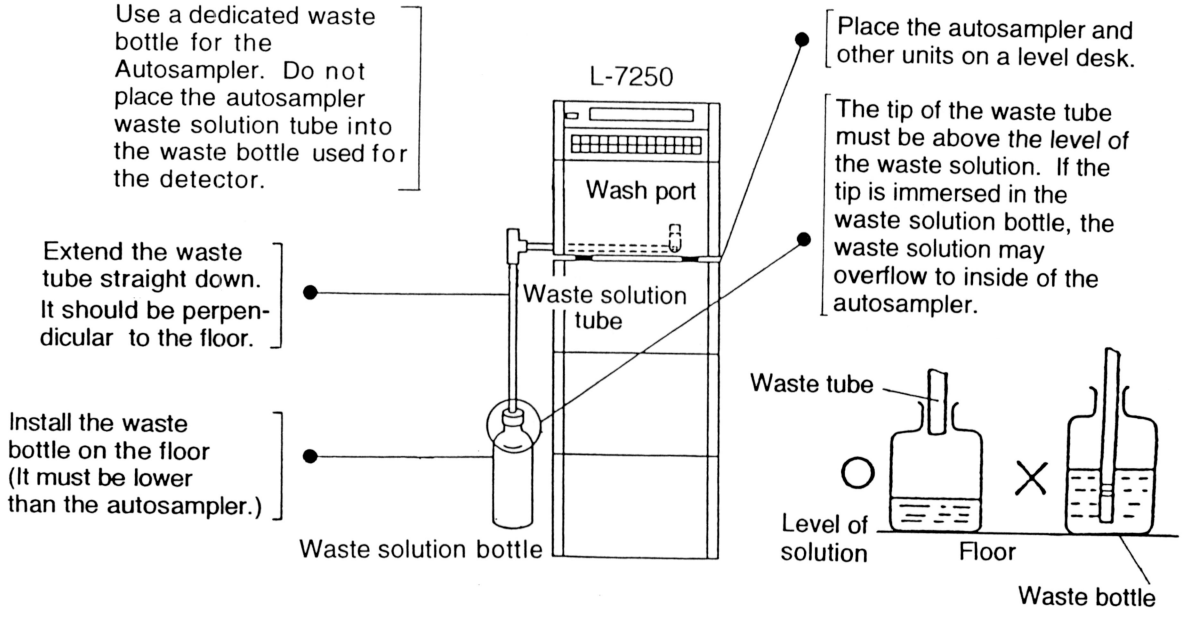


Figure 1-5 Waste Bottle and Waste Tube

2. Sample Vial Place the septum in the sample vials as shown in Figure 1-6.

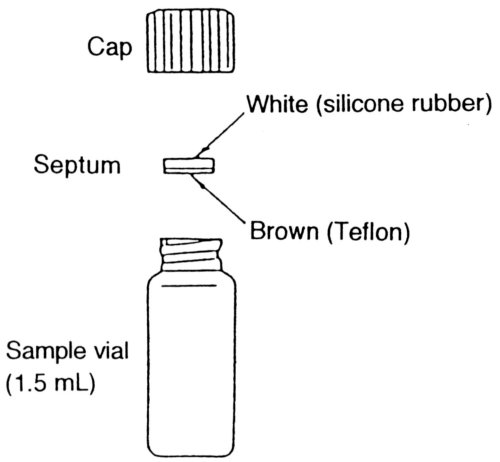


Figure 1-6 Components of the Sample Vial

3. Limitation on Composition of the Mobile Phase and Wash Solution.

[Important]

1. The column and the fittings (connectors) in the pump, autosampler, etc. are made of stainless steel (SUS316). Although this material is quite inert, halogen ions and other materials may corrode it. Avoid the use of a mobile phase that contains potentially corrosive materials. Table 1-1 is a listing of restricted reagents. Table 1-1 is not meant to be inclusive, if there is a concern about the possibility that a solution may corrode the fittings or the column, take a used fitting and place it in the solution for some time. Observe the effect on the fitting before using the solution in the system.
2. If it is necessary to use solutions which are potentially corrosive, wash the entire flow path with distilled water after finishing the analyses. Do not allow potentially corrosive mobile phases to remain in contact with the system for an extended time.

   Do not use mobile phase which contains salts that precipitate in the system as the percent of the organic modifier increases during a gradient. Thoroughly wash the entire flow path with distilled water after finishing any analyses in which the mobile phase contains any salts.

Table 1-1 Reagents which may Corrode Stainless Steel Fittings and the Column

Possible to Use	Possible to Use, If Less Than 50 Percent	Possible to Use, If Less Than 10 Percent	Should not be Used with SUS316
Phosphoric acid	Acetic acid	Dibasic sodium phosphate	Ammonium chloride
Sodium phosphate	Ammonium nitrate	Ammonium formate	Potassium chloride
	Ammonium nitrate	Ammonium perchlorate	Sodium chloride
	Citric acid	Ammonium phosphate	**Notes:** 1. Concentration of Reagents listed above should be 5-6 percent maximum.
	Butyric acid Sodium hydroxide	Boric acid Formic acid (up to pH3)	2. If you use the reagents listed above, be sure to wash the whole flow path with distilled water at the end of the analytical session.
	Sodium nitrate	Hydrochloric acid (up to pH3)	
		Potassium nitrate	
		Sodium bicarbonate	
		Sodium carbonate	

1.5 Routine Operations

⚠ WARNING

Ignition of Flammable Chemicals!

- Beware of ignition hazard when using flammable chemicals such as organic solvents.
- Always check the following conditions. If an abnormality is found, stop operation immediately.
  - ◇ Leakage of solvent or waste solution.
  - ◇ Leakage of solvent inside the instrument.
  - ◇ Inadequate ventilation of the laboratory room.
- This instrument is not explosion-proof. Although aqueous solvents or organic solvents having an ignition point of 70°C or higher are usable, do not use organic solvents having an ignition point below 70°C.
- When using flammable chemicals, be careful about possible ignition due to static electricity. Particularly when using non-conductive chemicals, employ a conductive vessel made of metal or the like and provide grounding connection correctly.

⚠ WARNING

Explosion of Vapor from Flammable Chemicals!

- If a flammable chemical such as organic solvent leaks from the flow path of the instrument and its vapor concentration exceeds the explosion limit, it may cause spontaneous combustion with dangerously explosive results.
- When using a flammable and readily volatile chemical, be sure to check for leakage from the instrument flow path and ventilate the laboratory room adequately.

⚠ CAUTION

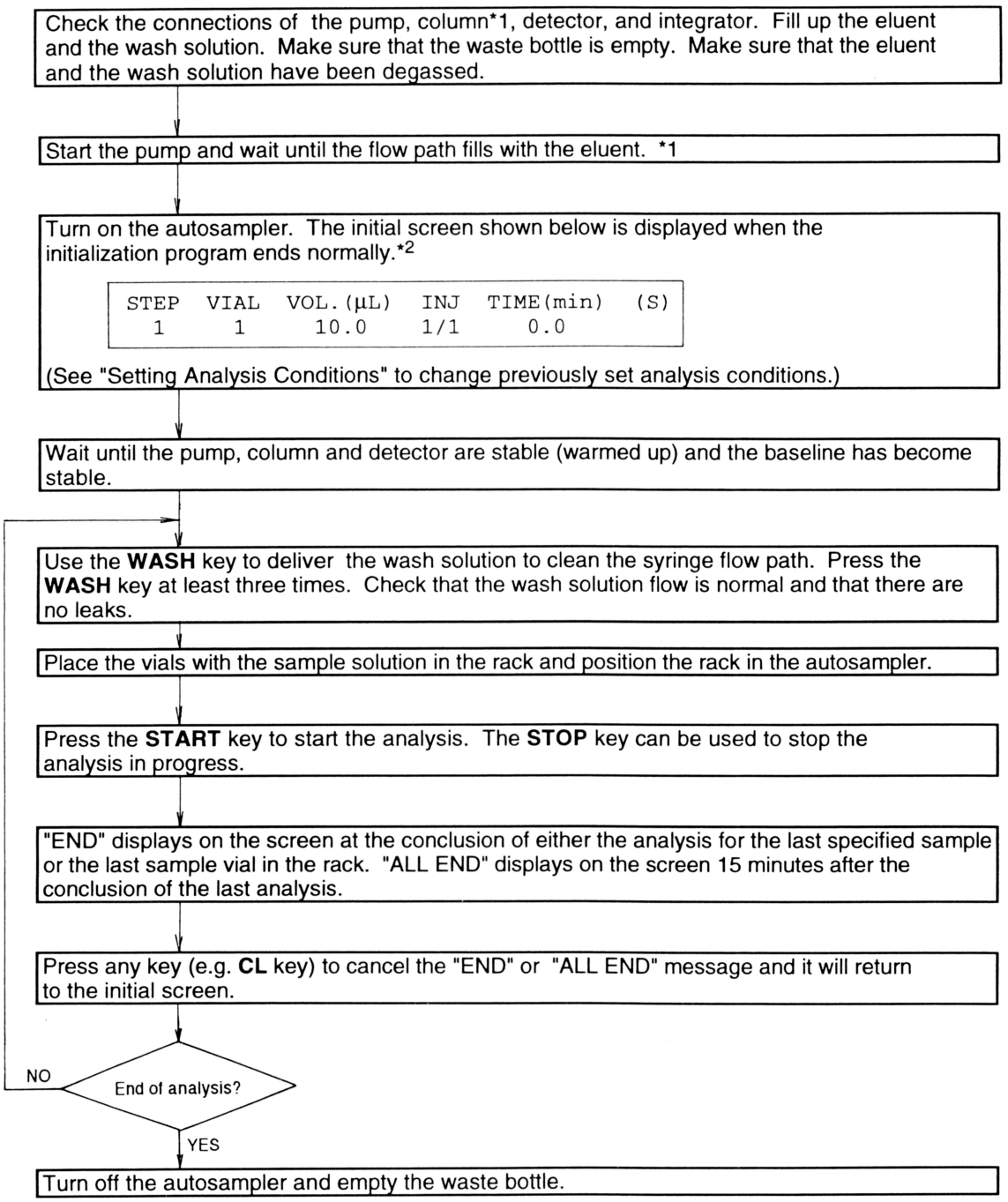
Be Cautious during Instrument Operation!

Never touch the inside of autosampler or put a thing into it during operation, as your hand or finger may be injured.

Be especially cautious when the arm, needle or syringe is in action.

When you power up the L-7250 Autosampler, the unit automatically recalls the analytical conditions which are stored in memory.

For routine operation, use the following procedure.



*1 Before installing a new column, ensure that the complete flow path is filled with eluent to prevent air from entering the column.

*2 When you select other than the sequential (programming) mode, a different screen displays. Pressing the **MODE** key calls up the sequential mode and displays the initial screen.

1.6 Setting of Simple Analysis Conditions

The next example describes the procedure for setting conditions for the sequential analysis of 120 samples with one 10 µL injection per sample. The autosampler will inject a sample every 10 minutes.

> **Note:** The next section shows use of the UTILITY key to set a number of instrumental parameters, such as the syringe speed, the cut volume, and the needle height.

In this discussion, information that is in bold type indicates data that the analyst enters. When you power on the unit, the autosampler goes through an initialization process. Upon completion of the initialization process after the power is turned on, the display shows the initial screen.

<Initial screen>

```
STEP  VIAL  VOL.(µL)  INJ  TIME(min)  (S)
  1     1     10.0    1/1     0.0
```

SET
PROG

After power on and upon completion of initialization, this screen appears.

```
CALIB (NO=0, YES=1)  NO. OF STD LEVELS
   0                          1
```

> **Note:** If you want to analyze a standard sample as part of the operation of the system, select the value 1 at this point. Section 2.3.2 discusses the use of the autosampler with standard (calibration) samples.

0 ENTER

```
NO.  VIALS  INJ VOL INJ/VIAL STOP TIME
 1   1-120      10      1         10
```

You can edit the number in the first field at this point.

1 ENTER

```
NO.  VIALS  INJ VOL  INJ/VIAL  STOP TIME
 1   1-120      10         1         10
```

Enter the number of the last vial you want to analyze.*

1 2 0 ENTER

```
NO.  VIALS  INJ VOL  INJ/VIAL  STOP TIME
 1   1-120      10         1         10
```

Enter the injection volume (in μL).

1 0 ENTER

```
NO.  VIALS  INJ VOL  INJ/VIAL  STOP TIME
 1   1-120      10         1         10
```

Enter the number of injections per vial.

1 ENTER

```
NO.  VIALS  INJ VOL  INJ/VIAL  STOP TIME
 1   1-120      10         1         10
```

Enter the analysis time (in minutes).

1 0 ENTER

```
LAST STEP (NO=0, YES=1)
    0
```

Since the operation you want requires only one set of 120 analyses, select YES

1 ENTER

If you select NO (0) and ENTER at this point, you could enter a set of different analysis conditions for the next operation of the autosampler. For additional information, refer to Section 2.3.2.

The analysis conditions are now set and the system displays the initial screen.

* Since the autosampler provides a vial detection function, you can enter the number of the last vial as a number that is higher than the number of samples that are in the rack. Usually, you select the maximum number of vials that can be placed on the rack as the number for the last vial. For a standard rack, this number is 120.

2 BASIC OPERATIONS

This chapter covers the basic operations of the L-7250 Autosampler when you operate it in the sequential mode. In this mode, the autosampler withdraws a sample from a vial in the rack and injects it into the mobile phase flow path. The order of injection is the order of vial placement in the sample rack.

2.1 Preparatory Operations

1. Check the autosampler connections to the pump and detector before turning on the power.

2. Check the drain tube connections to the waste bottle. The waste bottle must be at a level lower than the autosampler.

3. Ensure that the connections of all components are correct. Refer to the respective instrument manuals for additional information.

4. Place the detector drain tube in a dedicated waste bottle. If you place this drain tube in another waste bottle (e.g. the waste bottle for the autosampler), the back pressure will not be stable and you may observe fluctuations in the baseline.

5. Connect the eluent bottle to the pump and connect the wash solution bottle to the autosampler.

6. Start the pump.

NOTE: When you are using a new column, fill the tubing between the eluent bottle and the column inlet fittings with the degassed eluent (i.e. free of air bubbles). Do this before installing the column.

7. Turn on the autosampler.

   IMPORTANT:

   Do not turn on the autosampler until the eluent is at the injection valve of the autosampler. You may damage the injection valve seal if you switch the injection valve before the liquid reaches it.

   When the autosampler is powered up, initialization takes place automatically and the appropriate initial screen displays.

8. Clean the flow path (needle, syringe and tubing) before operation of the system. To clean the flow path, press the **WASH** key.

   Press the **WASH** key at least three times to guarantee that three wash cycles occur and to ensure the cleanliness of the flow path. Make sure that the liquid flows smoothly, that there are no leaks, and that the waste liquid tube flows into its own waste bottle.

Main Check Points for the System	
(1)	Pump • Ensure that the flow rate setting is correct • Check that you have chosen the appropriate pressure limit • Check that there is sufficient eluent in the eluent bottle
(2)	Autosampler • Check that there is sufficient wash solution in the wash solution bottle • Ensure that there is enough space in the autosampler waste solution bottle to accommodate the anticipated waste
(3)	Column • Ensure that the appropriate column is in place • Check that the maximum pressure is set on the pump module
(4)	Detector • Verify the correct wavelength setting • Ensure that there is enough space in the detector waste bottle to accommodate the eluent from the column
(5)	Integrator • Confirm that there is sufficient chart paper in the unit • Verify that you have the appropriate data processing parameters
(6)	Other Inspections • Check for leaks in the system (tighten joints if necessary) • Check for the presence of dried salts around joints (clean and tighten if necessary) • Ensure that there are no constrictions or obstructing bends in the tubing

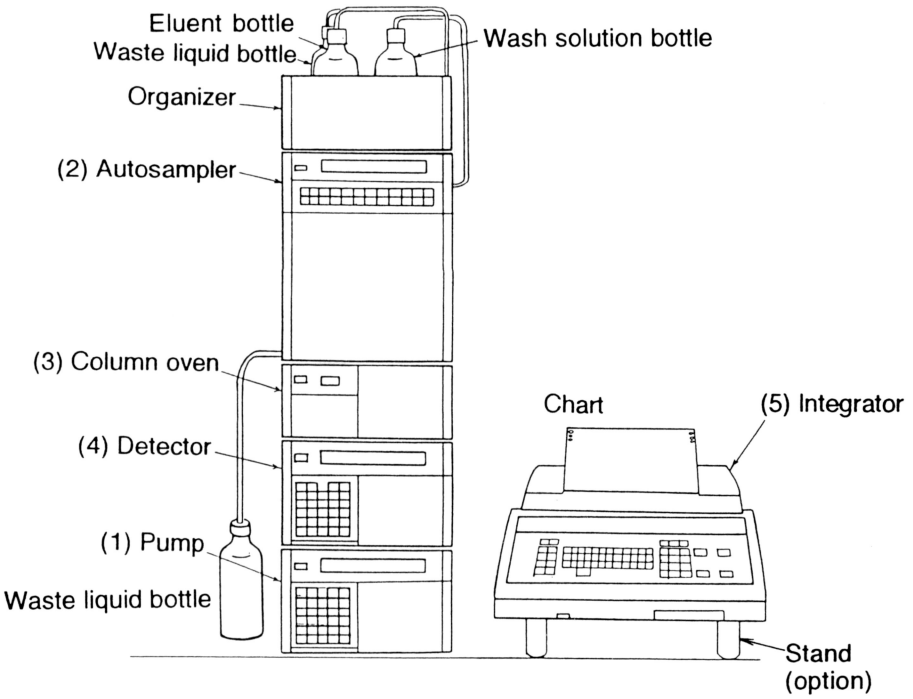


Figure 2-1 Example of the Liquid Chromatography System

2.2 Outline of System Operations

1. Initial Setting When you turn the power on, the autosampler goes through an initialization process. This initialization process includes an electronics test procedure and an automatic zero adjustment of the sampling mechanism. The system checks at this point the autosampler memory, the arm drive mechanism, the valve switch function, and other functions. At the end of each test, an OK message appears if its result is normal or an ERROR message if it is abnormal. When all the tests conclude, the initial screen displays if the system is functioning properly. For details on the significance of the ERROR messages, see Appendix: 1.

2. Overview of System Operations

Figure 2-2 is a flow chart of the various operational procedures in the sequential mode.

When you power on, the mode that was active when you turn off the power last is set up again.

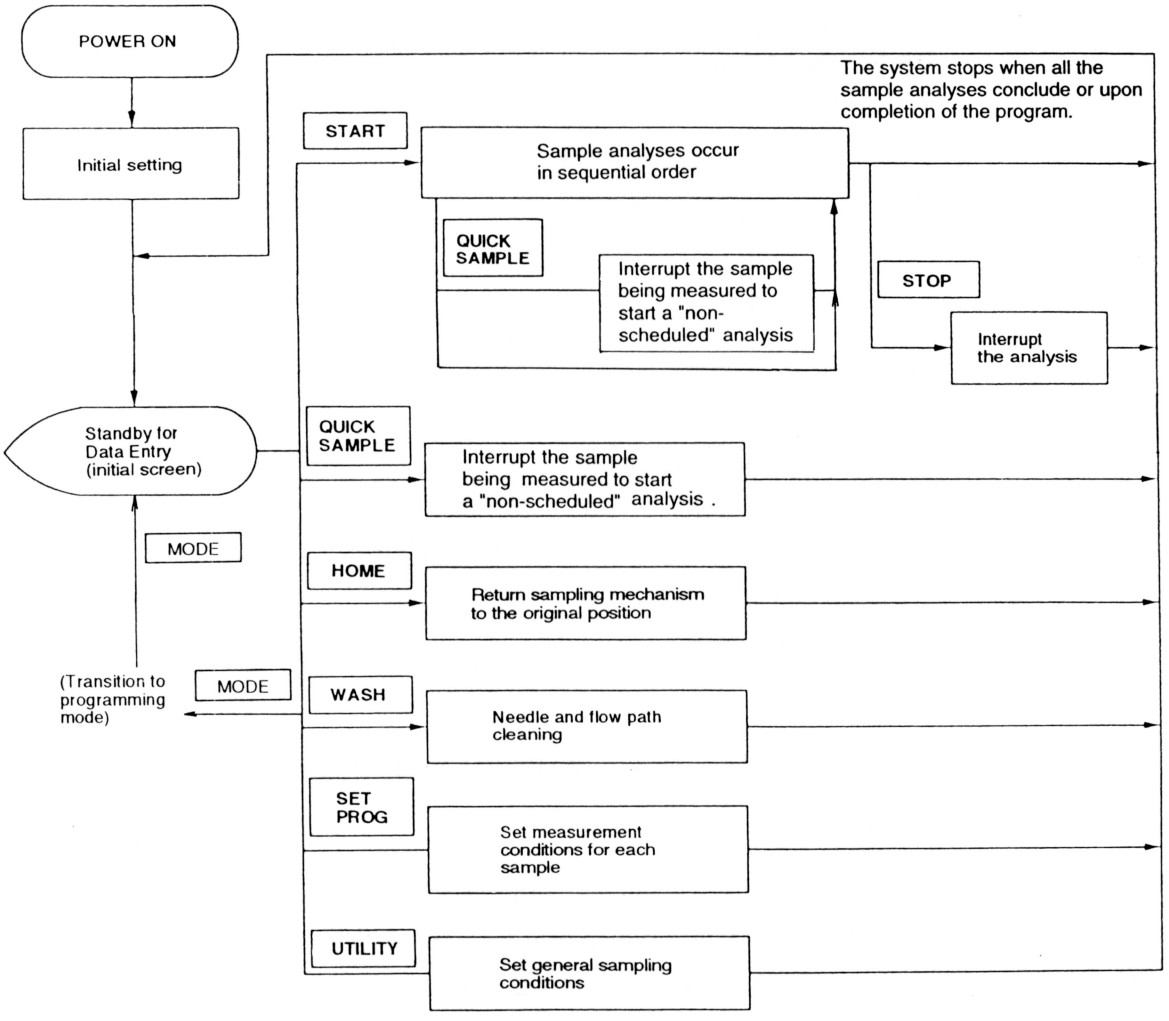


Figure 2-2 Operation Flow Chart

2.3 Operation of the Autosampler

2.3.1 Routine Operations

1. When the instrument completes the self test after power-on, described in Section 2.2, the initial screen of the sequential mode appears (see below). When you choose the programming mode or the external control mode, a screen different from this initial sequential mode screen appears. In this case, press the **MODE** key to set up the sequential mode and to reach the initial screen of the sequential mode. If you need conditions that are different from those used in the previous operation, follow the instructions that follow to change the conditions.

```
STEP  VIAL  VOL. (µl)  INJ  TIME(min)          (S)
1     1     10.0       1/1   0.0
```

(1) (2) (3) (4) (5) (6)

(1) A step in the running program
(2) The number of the vial that is being analyzed
(3) The amount of sample injected for this step.
(4) The number of times each sample has been injected/the desired number of injections.
(5) Analysis time
(6) Mode indications
[S]: Standard mode (cut injection method)
[A]: All volume injection mode (all volume injection method)
[F]: Full loop mode
[L]: Key lock mode
[H]: Hold mode
[R]: Analysis mode
[P]: Programming mode
[T]: Teach mode
[E]: Edit mode
[C]: External control mode

2. Wait until the pump, the column and the detector are stable (warmed up). Wait also for the baseline to become stable.

3. Press the **WASH** key. This action cleans the needle and the injection port and fills the syringe flow path with the wash solution.

4. Place the vials containing samples in the rack and position the rack in the autosampler.

5. Press the **START** key to start the analysis. You can monitor the progress of the analysis on the display.

6. When the analysis of all the vials on the rack or all the specified vials concludes, END displays on the screen. ALL END displays on the screen 15 minutes later.

7. Press any key (e.g. **CL** key) to cancel the END or ALL END message and return to the initial screen.

8. To continue analyzing other samples, press the **WASH** key to clean the entire flow path, install new samples and press the **START** key. If there are no other samples to analyze, turn off the power and empty the waste bottle. If the mobile phase contains reagents that might lead to corrosion (see Section 1.4.2), flush the system with water.

9. To terminate operation of the autosampler while a run is in progress, press the **STOP** key. This causes the analysis session to stop and the display returns to the initial screen.

2.3.2 Setting the Analysis Conditions

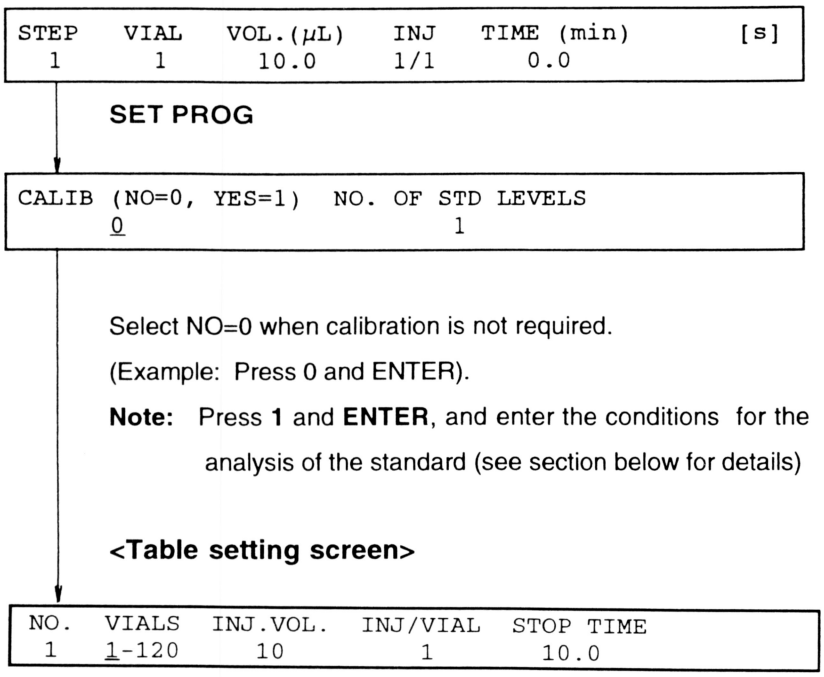
Use the **SET PROG** key to access the program and to enter the sampling conditions for each sample. Use the **UTILITY** key to access the program and to set the general operating conditions of the autosampler.

1. Setting the Analysis Conditions with the SET PROG Key

Use the **SET PROG** key to define the conditions for the sequential analysis of the samples present on the rack. You can establish conditions for each sample on an individual basis.

1a. To analyze unknown samples without measurement of standard solutions

<Initial screen>



Use this screen to set the analysis conditions for unknowns. The analysis table allows for up to 100 steps. You can specify the vial range and analysis conditions for each step. You can store the table in memory so that you do not need to set it up each time you power up the system. Figure 2-3 is an example of a multi-step analysis table.

(1)	(2)	(3)	(4)	(5)
NO.	VIALS	INJ.VOL.	INJ/VIAL	STOP TIME
1	1-30	10	1	10.0
2	31-40	20	3	30.0
3	41-50	10	5	50.0
.				
.				
.				
100	61-80	30	2	10.0

Figure 2-3 Example of a Multi-Step Analysis Table

(1) No. (Program step numbers)

The program step numbers appear in sequential order. Use the [△] and the [▽] keys to go to the next or the previous step. A program can include a total of 100 steps.

(2) VIALS (vial number)

VIALS displays the numbers of the first and last sample that you plan to analyze under the same conditions. The range is from 1 to 250. You can use up to 100 when using the standard rack.

(3) INJ.VOL. (injection volume)

You can set the injection volume between 0 µl and 400.0 µl. The sample volume should be at least 10 µl as the accuracy of analysis degrades if the volume is too small. The range of the sample volumes that you can select depends on the capacity of the syringe.

(4) INJ/VIAL (injections/vial: the number of injections per vial)

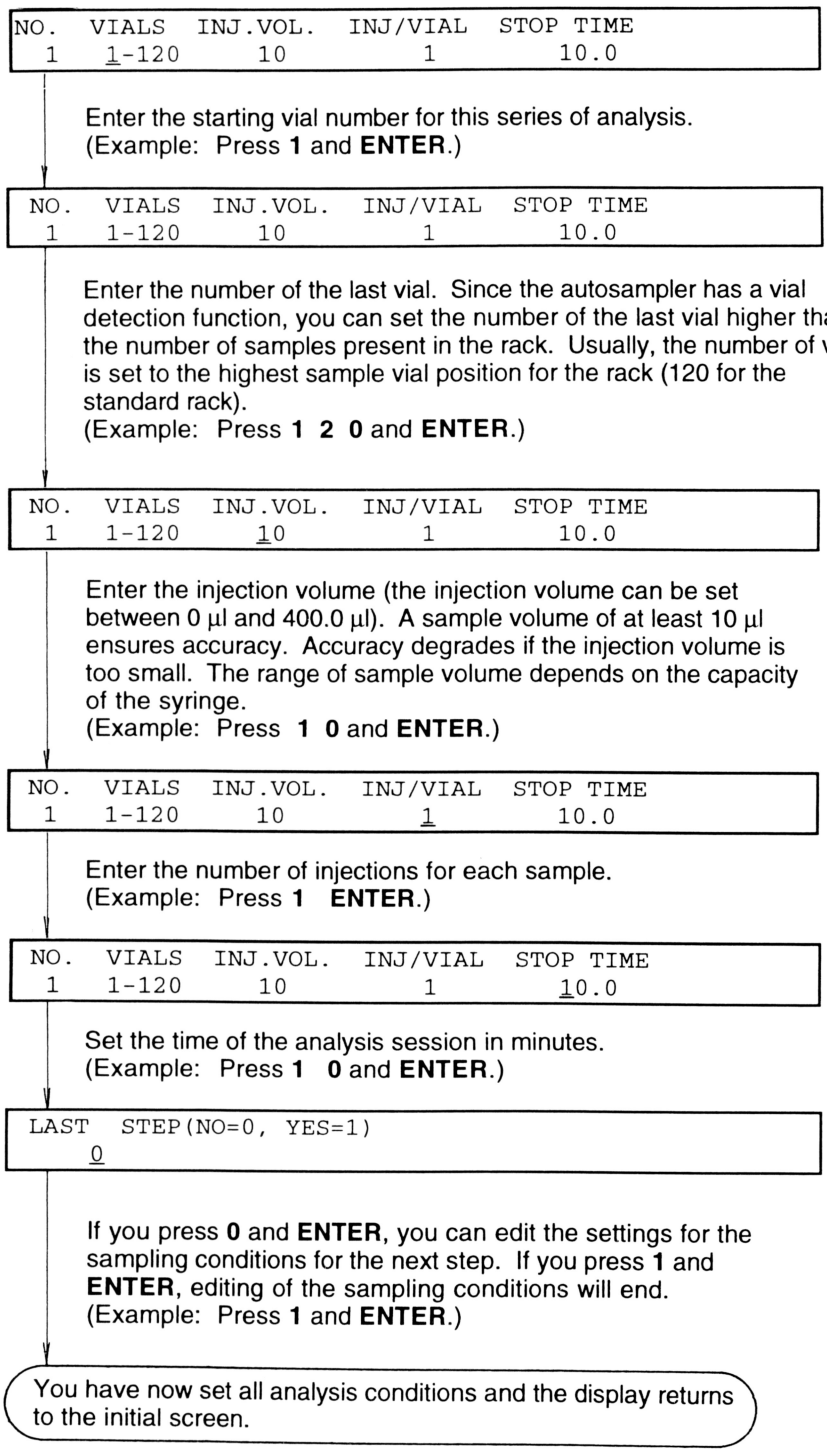
Enter the number of injections that are to be made for each sample. Set a number between 1 and 99.

(5) STOP TIME (end of analysis session)

Set the time for the analysis. You can enter any time up to 999.9 minutes. The sample time count starts when the sample injects. At the end of the analysis time, the next sample injects. If the actual operating time of the HPLC is greater than the set analysis time on the autosampler, a minimum amount of time will take place after the end of the analysis before the next injection (i.e. there will be no scheduled waiting period).

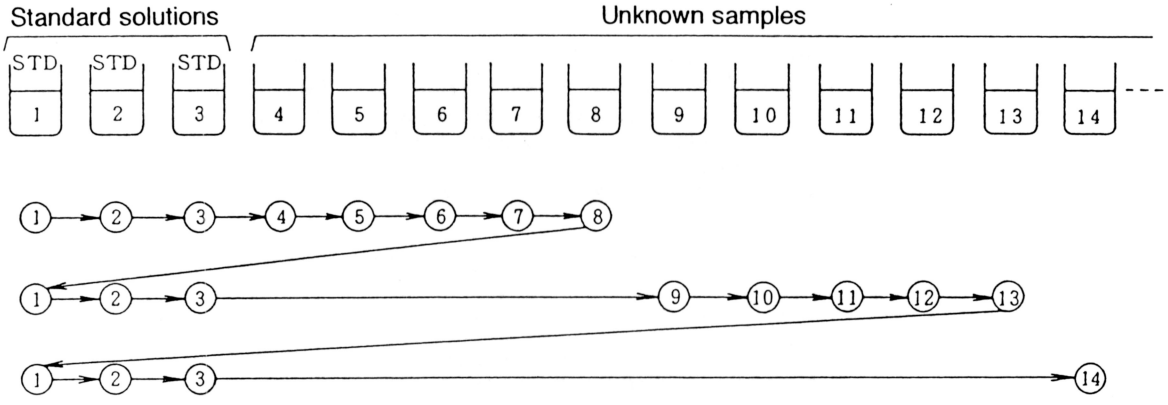
The following example shows how to set sampling conditions in the analysis table.

<Table setting screen>



1b. Periodic analysis of standard samples

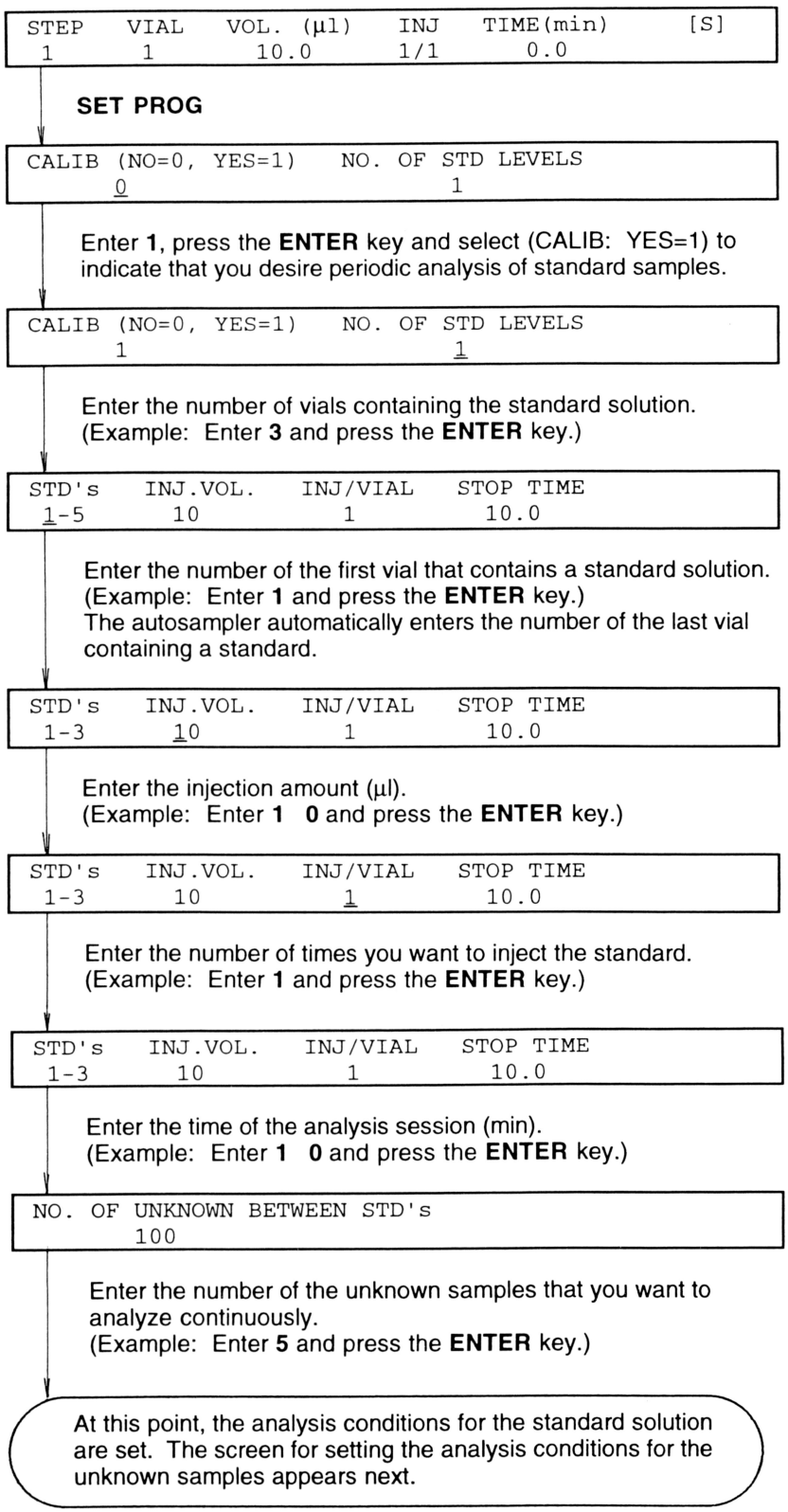
In some analyses, you may want to periodically analyze a standard (a sample in which the concentration of the compound(s) of interest is known). In this situation, you need to enter the sampling conditions for the standard solution. The next procedure describes an example in which there are 3 vials containing the standard solutions. The procedure calls for you to measure the standard solutions after every 5 unknown samples.



When the system starts, it analyzes three standard solutions first and then five unknown samples. After the analysis of the fifth sample, the autosampler injects the three standard solutions again. This process continues until the analysis of all samples concludes.

Figure 2-4 Example Showing the Analysis Sequence of the Standard Solutions and Unknown Samples

<Initial screen>



The number of the first unknown sample is the number of the vial after the last standard solution. Since there are three standard samples in this example, the number of the first unknown sample is 4.

```
NO.    VIALS    INJ.VOL.    INJ/VIAL    STOP TIME
 1     4-120       10           1          10.0
```

2. Setting General Sampling Conditions with the UTILITY Screen Use the **UTILITY** key to display the screen that lets you specify a variety of general operating conditions.

<Initial screen>

```
STEP    VIAL    VOL.(µl)    INJ    TIME (min)        [S]
  1       1       10.0      1/1       0.0
```

UTILITY

< Screen for setting operating conditions >

```
[UTILITY] 1
1)SEQ  2)RACK PARAM  3)APL PARAM  4)OPTION
```

Select the item on this screen to specify the conditions you want to edit.

2a. Operating conditions (SEQ) To specify operating conditions, select "1) SEQ" on the utility screen shown above.

<UTILITY screen>

```
[UTILITY] 1
1)SEQ  2)RACK PARAM  3)APL PARAM  4)OPTION
```

1 ENTER

```
UTILITY/SEQ 1
1)START STEP  2)OPERATION PARAM  3)ALL END
```

1 ENTER

```
UTILITY/SEQ/START STEP
START STEP (1-100)=1
```

Enter the starting step. Use the START STEP when you want to start the analysis midway in the analysis table rather than at step 1. (Example: Enter **1** and press the **ENTER** key.)

Note: To reset the value for the start step to 1 after the analysis concludes, press the **START** key. When you specify standard solution measurements, the autosampler will inject unknown samples after the measurements of the standards.

Returns to the initial screen.

2 ENTER

```
UTILITY/SEQ/OPERATION 1
1)RACK SELECT  2)SYR SPEED  3)INJ.METHOD
```

Select the operating conditions.

1 ENTER

```
UTILITY/SEQ/OPERATION/RACK SELECT
RACK CODE (1-16)=1
```

Select the vial rack you plan to use. You can store information about 16 rack types. (Use the RACK PARAM function to set the information about the rack configuration.)
(Example: Enter **1** and press the **ENTER** key.)

Returns to the initial screen.

2 ENTER

```
UTILITY/SEQ/OPERATION/SYR SPEED
SYRINGE SPEED (1-5)=5
```

Set the syringe speed. Speed "1" is the most usual. Reduce the syringe speed as the degree of the sample viscosity increases. Table 2-1 shows the flow rates for a 0.5 mL syringe . A syringe speed for washing should be set at **2**.
(Example: Enter **1** and press the **ENTER** key.)

Return to the initial screen.

Table 2-1 Syringe Speed Number and Flow Rate for the 500 µl Syringe

Speed Number	Flow Rate (µl/s)
1	5
2	10
3	37.5
4	75
5	150

3 ENTER

```
UTILITY/SEQ/OPERATION/INJ METHOD
INJ. METHOD NO.(1-3)=1 (1:CUT 2:ALL 3:LOOP)
```

Select the method of a sample injection. (See section 3-2-3)
1 ENTER (Cut injection method)

```
UTILITY/SEQ/OPERATION/INJ METHOD/CUT
LEAD VOLUME (0-1000 µl)=30.0
```

Enter the lead volume*. (See Figure 3-2)
(Example: Enter **3 0** and press the **ENTER** key)

```
UTILITY/SEQ/OPERATION/INJ METHOD/CUT
REAR VOLUME (0-1000 µl)=30.0
```

Enter the rear volume*. (See Figure 3-2)
(Example: Enter **3 0** and press the **ENTER** key.)

Returns to the initial screen.

Normally, you should enter each of the cut volumes to about 30 µl for each .
You may have to reduce the cut volume for small sample volumes.

Note: The reproducibility may degrade if the cut volume is set to 10 µl or less. The cut volume influences dilution and also the absolute analysis value. (See section 2.5.1)

2 ENTER (All volume injection method)

```
UTILITY/SEQ/OPERATION/INJ METHOD/ALL
FEED VOLUME (0-1000 µl)=30.0
```

* See section 3.2.2 for further information on lead volume, rear volume and feed volume.

Enter the feed volume*. The feed volume should be set at about 30 µl for a 10 µl sample injection. (See section 2.5.3)
(Example: Enter **3 0** and press the **ENTER** key.)

Returns to the initial screen.

3 ENTER

```
UTILITY/SEQ/OPERATION/INJ METHOD/LOOP
WASTE VOLUME (0-400 µl)=400
```

Specify the waste volume. Typically, you should set the waste volume at about 100 µl for a 10 µl sample injection.
(Example: Enter **1 0 0** and press the **ENTER** key.)

Returns to the initial screen.

3 ENTER

```
UTILITY/SEQ/ALL END
ALL END OUT (0-1) = 0 (0:NO 1:YES)
```

You can output an ALL END signal when measurement ends in the sequential mode due to detection of an empty vial, etc. You can use this signal to control another module connected with the system.
Enter "1" (YES) to output the ALL END signal, or "0" (NO) when it is unnecessary.
(Example: Enter **1** and press the **ENTER** key.)
The delay time entry screen will now appear.

```
UTILITY/SEQ/ALL END
ALL END TIME (0.1-100.0 min) = 15.0
```

You can set the delay(wait) time from the occurrence of END status until the ALL END signal is output. This allows you to set a certain time between washing the system with the pump, for example, and stopping the system. This delay time is settable in a range of 0.1 to 100 minutes. Enter a time of 20 minutes as follows:
(Example: Enter 2 0 and press the **ENTER** key.)

Returns to the initial screen.

NOTE: You can output the ALL END signal only when the communication mode of L-7250 is set to the RS-232C mode.

2b. Rack parameters (RACK PARAM) Rack parameters indicate the rack dimensions, the number of test tubes that the rack can accommodate, the needle height, and the sampling pattern.

2b1. Rack parameter dimensions

X1 : X coordinate for the first tube (0-156.0 mm)
Y1 : Y coordinate for the first tube (0-156.0 mm)
X2 : X coordinate for the last tube (0-156.0 mm)
Y2 : Y coordinate for the last tube (0-156.0 mm)
Z1 : Needle height (Distance from the top point) (0-57.0 mm)
Nx : Number of tubes in the X axis direction (1-20)
Ny : Number of tubes in the Y axis direction (1-20)
P : Sampling Order Pattern Code (1-16), see Figure 2-6.

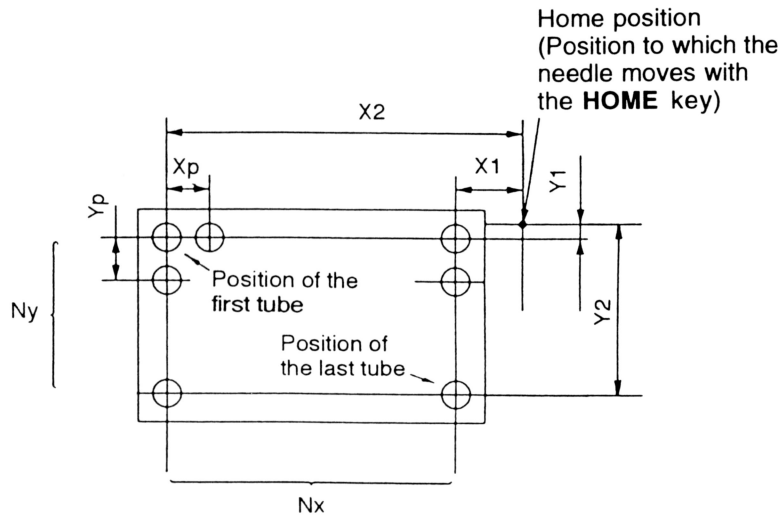


Figure 2-5 Rack Parameter Setting

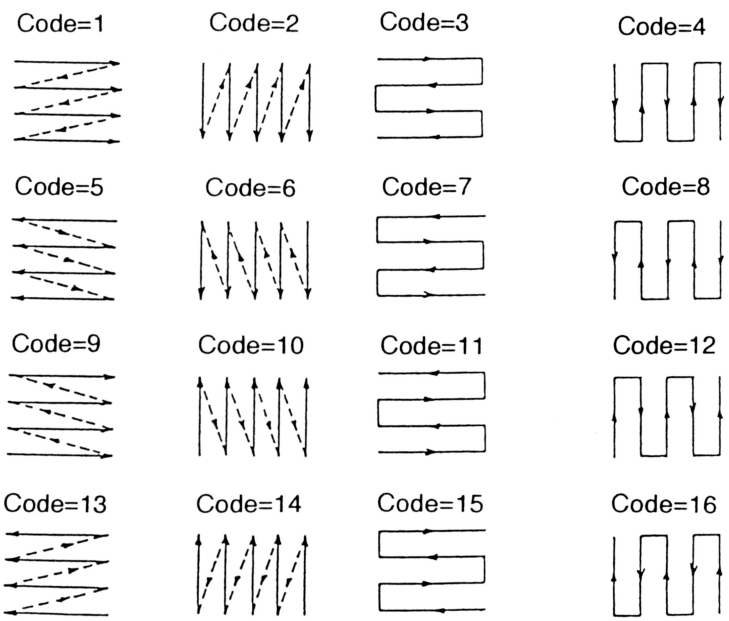


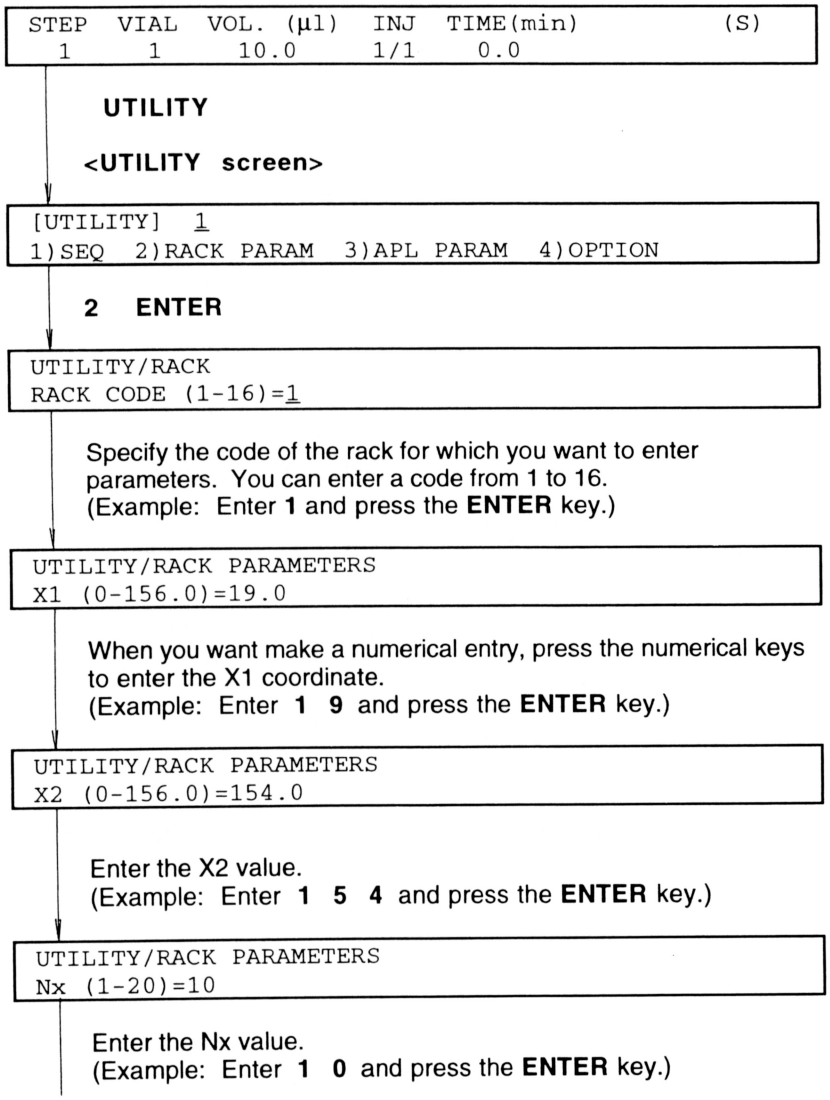
Figure 2-6 Sampling Order Pattern Codes

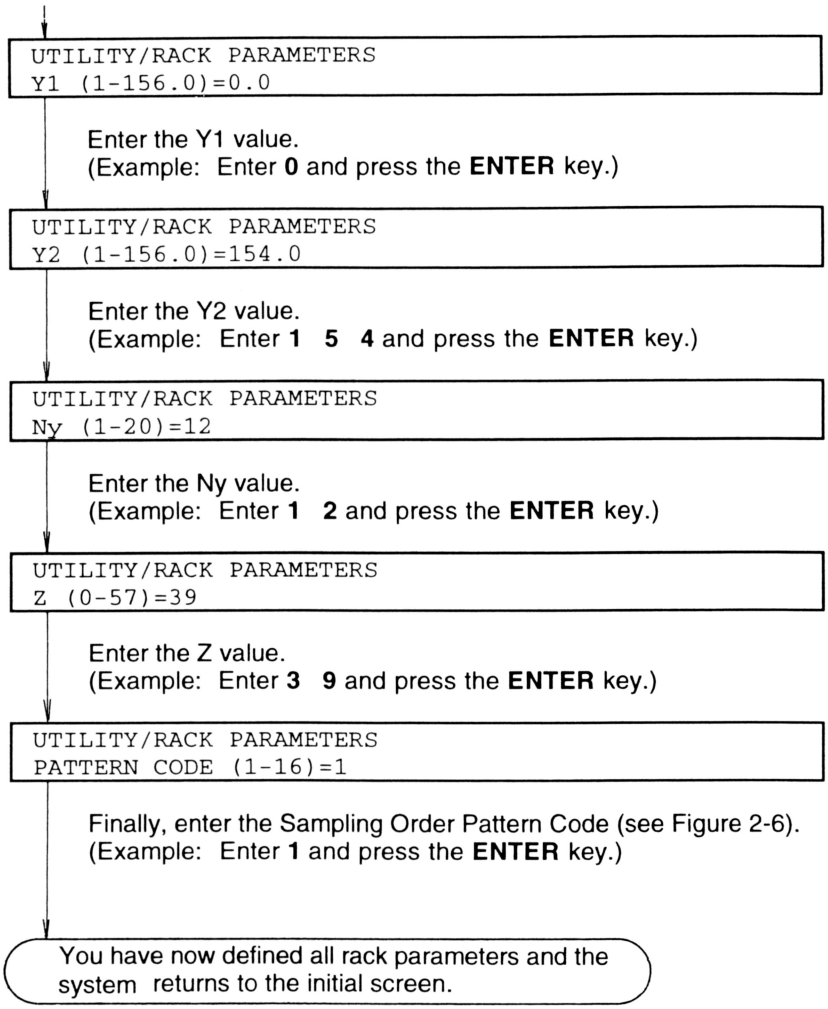
2b2. Procedures for setting the rack parameters

You can use two procedures to set the rack parameters. In one procedure, you enter numeric values. This procedure is convenient when you already know the rack size. In the other procedure (robotic mode setting), move the needle to an actual position for setting up its coordinate location. You can enter rack parameters by selecting "2) RACK PARAM" on the UTILITY screen.

• Setting the rack parameters by entering numeric values

<Initial screen>




UTILITY/RACK PARAMETERS
Y1 (1-156.0)=0.0
Enter the Y1 value.
(Example: Enter 0 and press the ENTER key.)
UTILITY/RACK PARAMETERS
Y2 (1-156.0)=154.0
Enter the Y2 value.
(Example: Enter 1 5 4 and press the ENTER key.)
UTILITY/RACK PARAMETERS
Ny (1-20)=12
Enter the Ny value.
(Example: Enter 1 2 and press the ENTER key.)
UTILITY/RACK PARAMETERS
Z (0-57)=39
Enter the Z value.
(Example: Enter 3 9 and press the ENTER key.)
UTILITY/RACK PARAMETERS
PATTERN CODE (1-16)=1
Finally, enter the Sampling Order Pattern Code (see Figure 2-6).
(Example: Enter 1 and press the ENTER key.)
You have now defined all rack parameters and the system returns to the initial screen.

• Setting the rack parameters in the robotics mode

Note: The needle may be damaged if it is moved laterally while it is down. When setting rack parameters in the robotic mode, you can move the needle laterally while it is down. You can also move the needle laterally during program execution. So in the robotic mode or during program execution, be careful not to execute the MOVE command while the needle is down.

<Initial screen>

```
STEP  VIAL  VOL(µl)  INJ  TIME (min)          [S]
 1     1     10.0    1/1     0.0
```

UTILITY

<Utility screen>

```
(UTILITY) 1
1)SEQ  2)RACK PARAM  3)APL PARAM  4)OPTION
```

2 ENTER

```
UTILITY/RACK
RACK CODE (1-16)=1
```

Enter the rack code in the range of 1 to 16. The needle moves to the home position when you enter the rack code.
(Example: Enter **1** **6** into the numerical keyboard and press the **ENTER** key.)

```
UTILITY/RACK PARAMETERS
X1 (0-156.0)=19.0
```

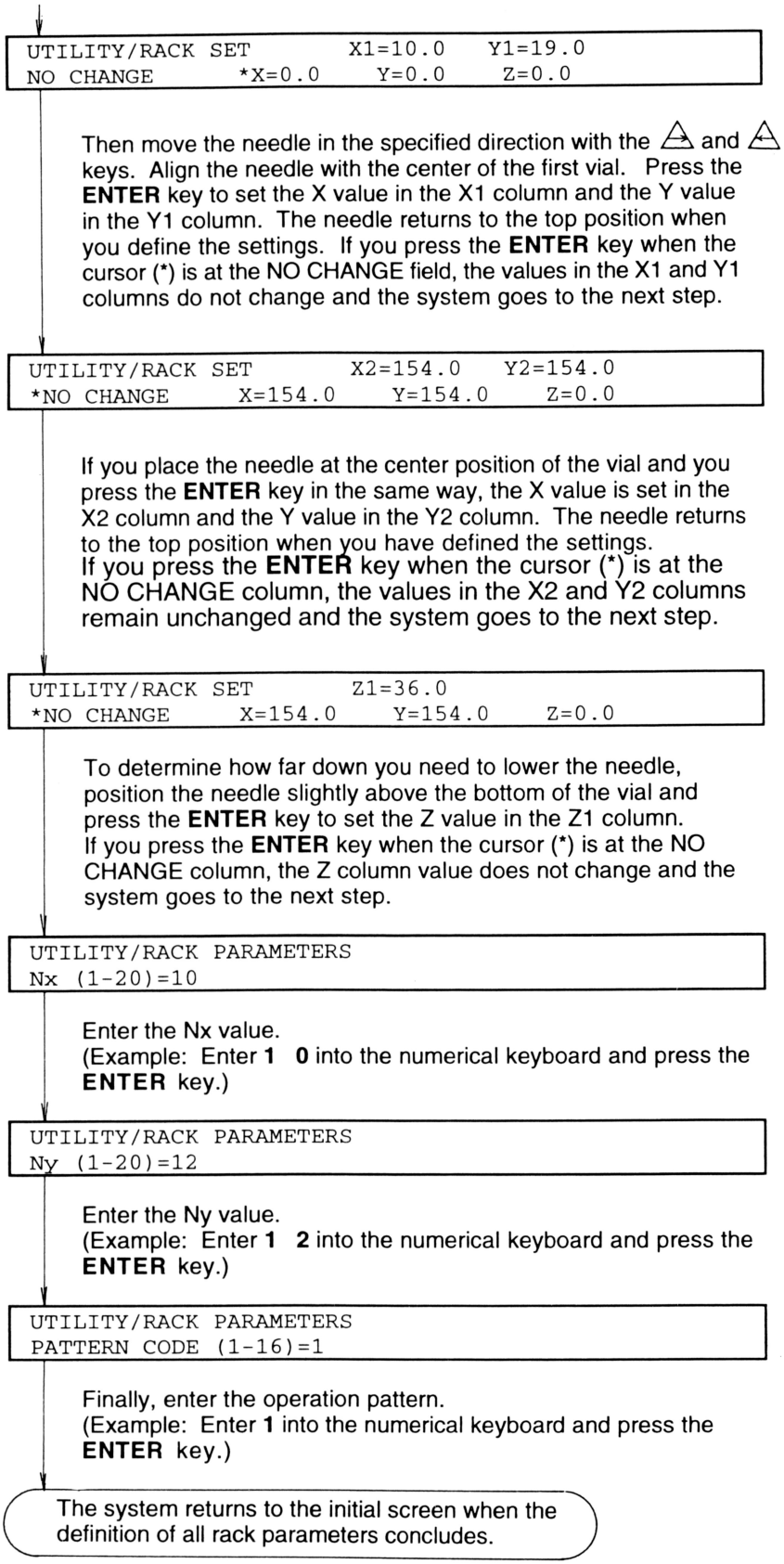
Use the ▽ and △ keys to move the needle to the actual position of a rack. These arrow keys make it possible to set the rack parameters in the robotics mode.
(Example: Press ▽ or △ and the **ENTER** key.)

Previously set values

```
UTILITY/RACK SET        X1=10.0    Y1=19.0
*NO CHANGE        X=0.0     Y=0.0     Z=0.0
```

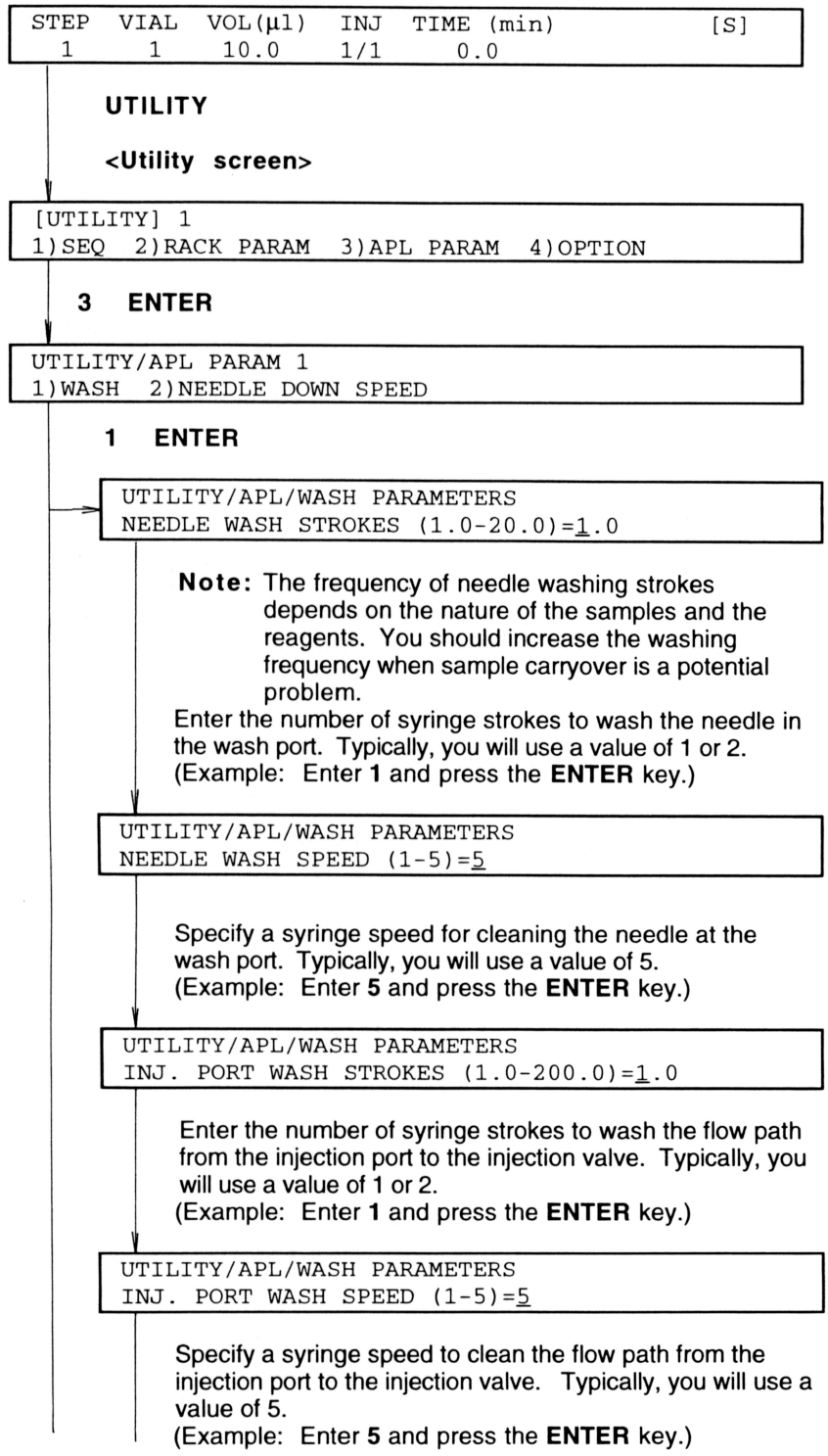
Currently set values

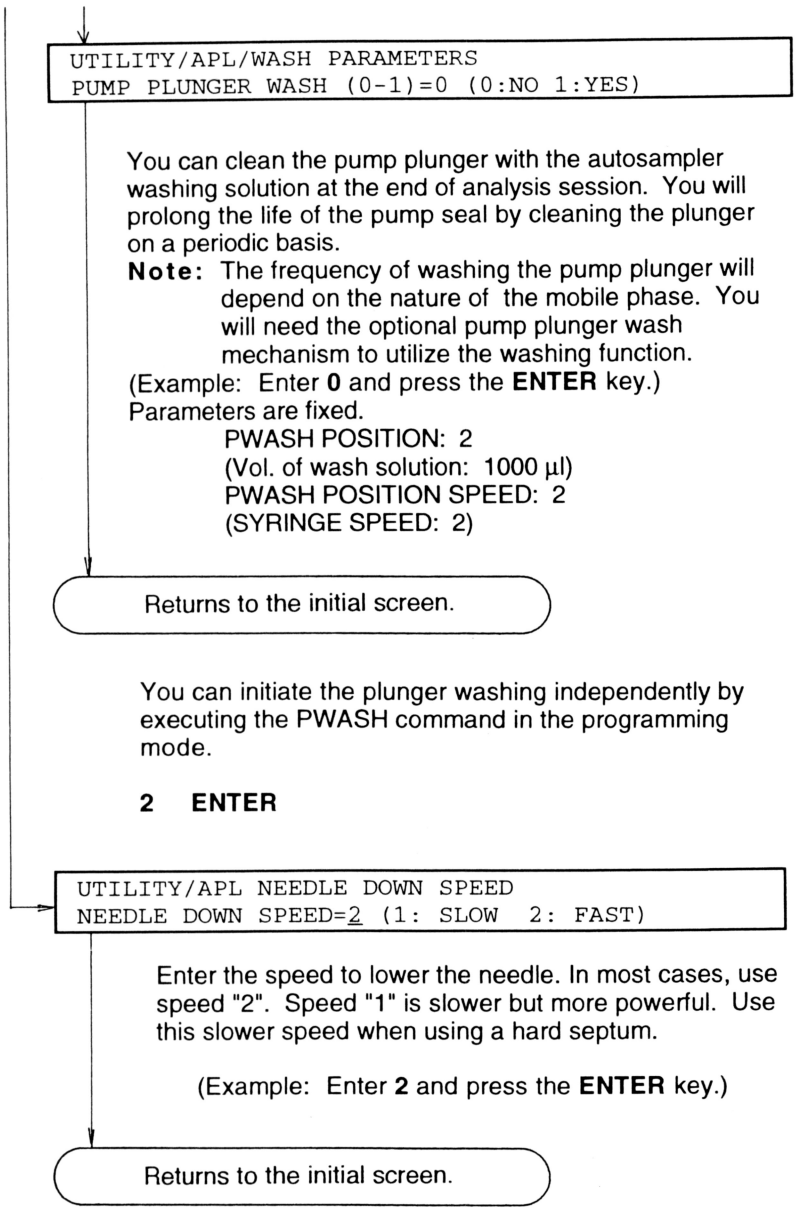
Specify the direction to move the needle with the **SELECT** key. The cursor (*) appears at a currently set direction.


UTILITY/RACK SET X1=10.0 Y1=19.0
NO CHANGE *X=0.0 Y=0.0 Z=0.0
Then move the needle in the specified direction with the and keys. Align the needle with the center of the first vial. Press the ENTER key to set the X value in the X1 column and the Y value in the Y1 column. The needle returns to the top position when you define the settings. If you press the ENTER key when the cursor (*) is at the NO CHANGE field, the values in the X1 and Y1 columns do not change and the system goes to the next step.
UTILITY/RACK SET X2=154.0 Y2=154.0
*NO CHANGE X=154.0 Y=154.0 Z=0.0
If you place the needle at the center position of the vial and you press the ENTER key in the same way, the X value is set in the X2 column and the Y value in the Y2 column. The needle returns to the top position when you have defined the settings.
If you press the ENTER key when the cursor (*) is at the NO CHANGE column, the values in the X2 and Y2 columns remain unchanged and the system goes to the next step.
UTILITY/RACK SET Z1=36.0
*NO CHANGE X=154.0 Y=154.0 Z=0.0
To determine how far down you need to lower the needle, position the needle slightly above the bottom of the vial and press the ENTER key to set the Z value in the Z1 column.
If you press the ENTER key when the cursor (*) is at the NO CHANGE column, the Z column value does not change and the system goes to the next step.
UTILITY/RACK PARAMETERS
Nx (1-20)=10
Enter the Nx value.
(Example: Enter 1 0 into the numerical keyboard and press the ENTER key.)
UTILITY/RACK PARAMETERS
Ny (1-20)=12
Enter the Ny value.
(Example: Enter 1 2 into the numerical keyboard and press the ENTER key.)
UTILITY/RACK PARAMETERS
PATTERN CODE (1-16)=1
Finally, enter the operation pattern.
(Example: Enter 1 into the numerical keyboard and press the ENTER key.)
The system returns to the initial screen when the definition of all rack parameters concludes.

2c. Setting the application operation conditions (APL PARAM) Select the "3) APL PARAM" on the utility screen to set the application operating conditions.

<Initial screen>





Important:

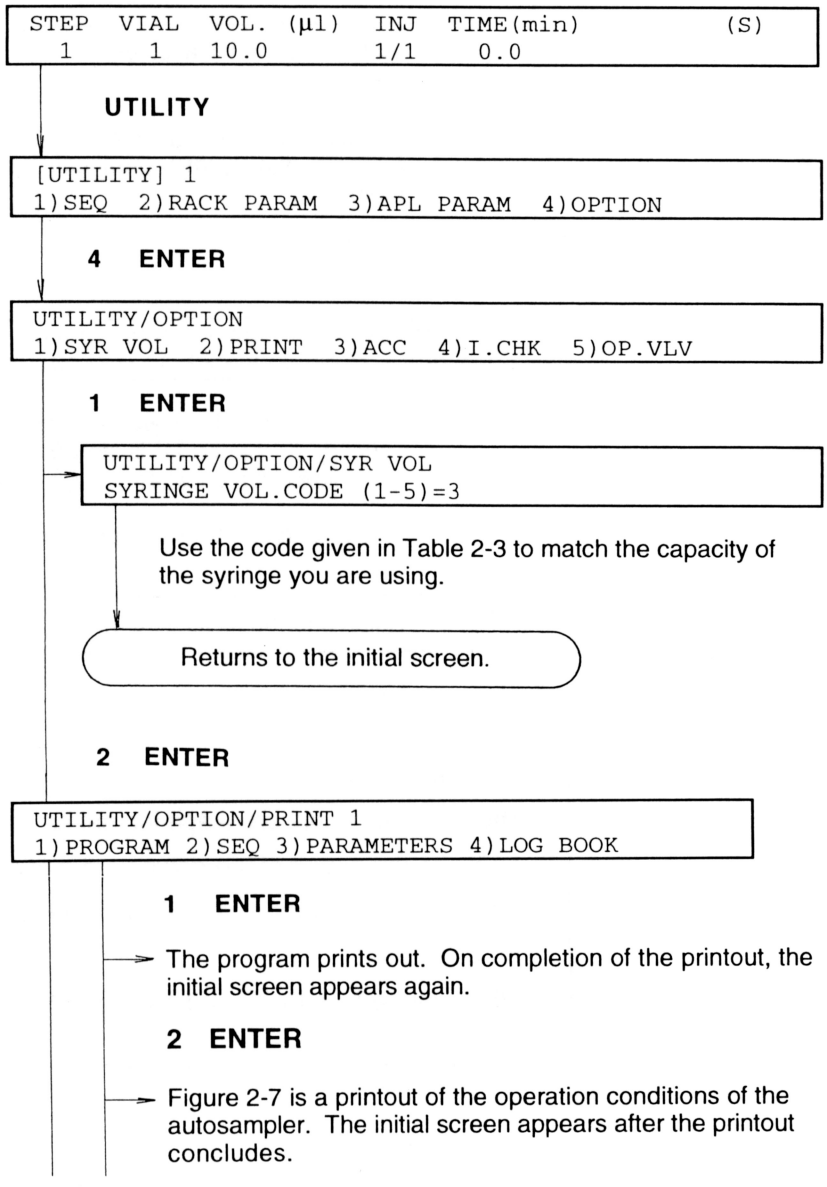
1. The risk of a needle breakage increases at low needle speed because the power of the downward motion increases. Ensure that the sample vial installation is correct before starting an analysis.
2. When you reduce the speed, the sound of the autosampler mechanism increases.

Table 2-2 Downward Speed of the Needle

Code	Type	Speed
1	SLOW speed	5.4 mm/s
2	FAST speed	72 mm/s

2d. Setting optional operating conditions (OPTION) Select "4) OPTION" on the utility screen to select the optional operating conditions.

<Initial screen>



3 ENTER

→ Figure 2-8 is a printout of the parameter list and rack code information. The initial screen appears after the printing concludes.

4 ENTER

→ Figure 2-9 is a printout of the logbook data. The initial screen appears after the printing concludes.

Note: The print function is available only when you connect Model D-7500 Integrator through the D-line communication interface.

3 ENTER, 4 ENTER, 5 ENTER

→ (Accessory: Specify ACC when this accessory is present. Refer to the information for this accessory.)

Table 2-3 Syringe Codes

Capacity	Code
0.1 ml	1
0.5 ml	2
1.0 ml	3
2.5 ml	4
5.0 ml	5

Note: Syringe Speed and Pressure Resistance. Table 2-4 shows the relationship between syringe speed and the flow rate. The "unusable" fields in the table indicate that the syringe inner pressure exceeds the syringe pressure resistance.

Table 2-4 Syringe Speed and Flow Rate

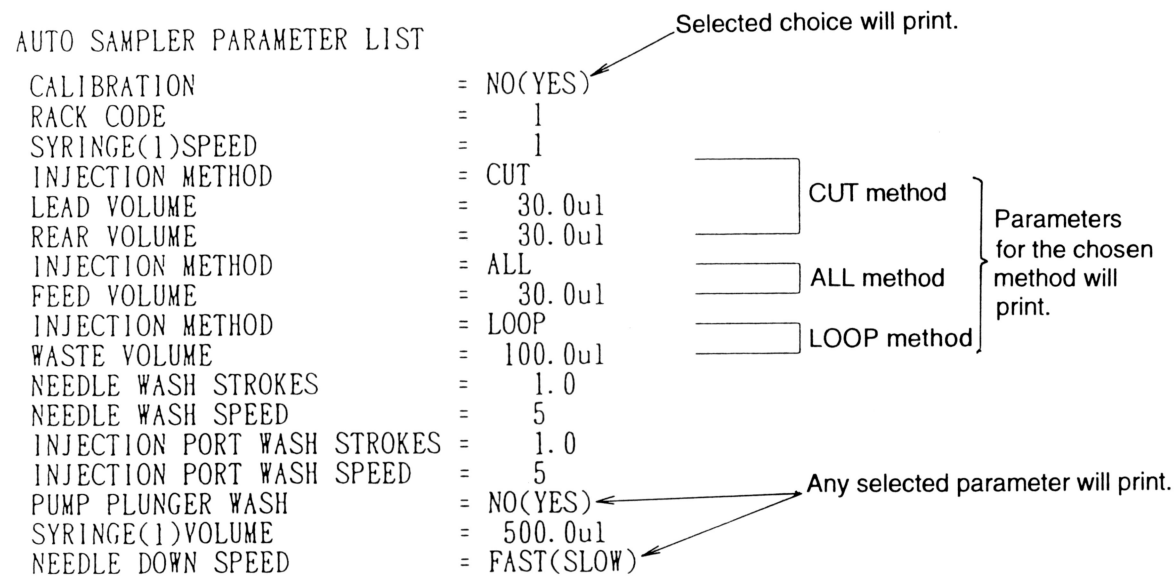
	Flow Rate (µl/s)				
Syringe Capacity	**0.1 ml**	**0.5 ml**	**1.0 ml**	**2.5 ml**	**5 ml**
Syringe speed 1	1	5	10	25	50
Syringe speed 2	2	10	20	50	100
Syringe speed 3	7.5	37.5	75	187.5	Un-usable
Syringe speed 4	15	75	150	Un-usable	Un-usable
Syringe speed 5	30	150	300	Un-usable	Un-usable

AUTO SAMPLER SEQUENTIAL TABLE LIST

STD'S	INJ.VOL.(ul)	INJ/VIAL	STOP TIME(min)
1- 2	10.0	1	10.0

NO.	VIALS	INJ.VOL.(ul)	INJ/VIAL	STOP TIME(min)
1	1- 30	10.0	1	10.0
2	31- 50	10.0	2	30.0

Figure 2-7 Sequential Table



RACK CODE	X1	X2	Nx	Y1	Y2	Ny	Z	P
1	19.0	154.0	10	0.0	154.0	12	39.0	1
2	19.0	154.0	10	0.0	154.0	12	39.0	1
3	19.0	154.0	10	0.0	154.0	12	39.0	1
4	19.0	154.0	10	0.0	154.0	12	39.0	1
5	19.0	154.0	10	0.0	154.0	12	39.0	1
6	19.0	154.0	10	0.0	154.0	12	39.0	1
7	19.0	154.0	10	0.0	154.0	12	39.0	1
8	19.0	154.0	10	0.0	154.0	12	39.0	1
9	19.0	154.0	10	0.0	154.0	12	39.0	1
10	19.0	154.0	10	0.0	154.0	12	39.0	1
11	19.0	154.0	10	0.0	154.0	12	39.0	1
12	19.0	154.0	10	0.0	154.0	12	39.0	1
13	19.0	154.0	10	0.0	154.0	12	39.0	1
14	19.0	154.0	10	0.0	154.0	12	39.0	1
15	19.0	154.0	10	0.0	154.0	12	39.0	1
16	19.0	154.0	10	0.0	154.0	12	39.0	1

Figure 2-8 Parameter List Printout

```
AUTO SAMPLER LOGBOOK

 PROGRAM NO.                      = 8108350-01
 INJECTION PORT SEAL  (times)     = 99999
              (DATE:MM/DD/YY)     =  1/ 1/94
 INJECTION VALVE SEAL (times)     = 99999
              (DATE:MM/DD/YY)     =  1/ 1/94
 SYRINGE              (times)     = 99999
              (DATE:MM/DD/YY)     =  1/ 1/94
 SVALVE SEAL          (times)     = 99999
              (DATE:MM/DD/YY)     =  1/ 1/94
```

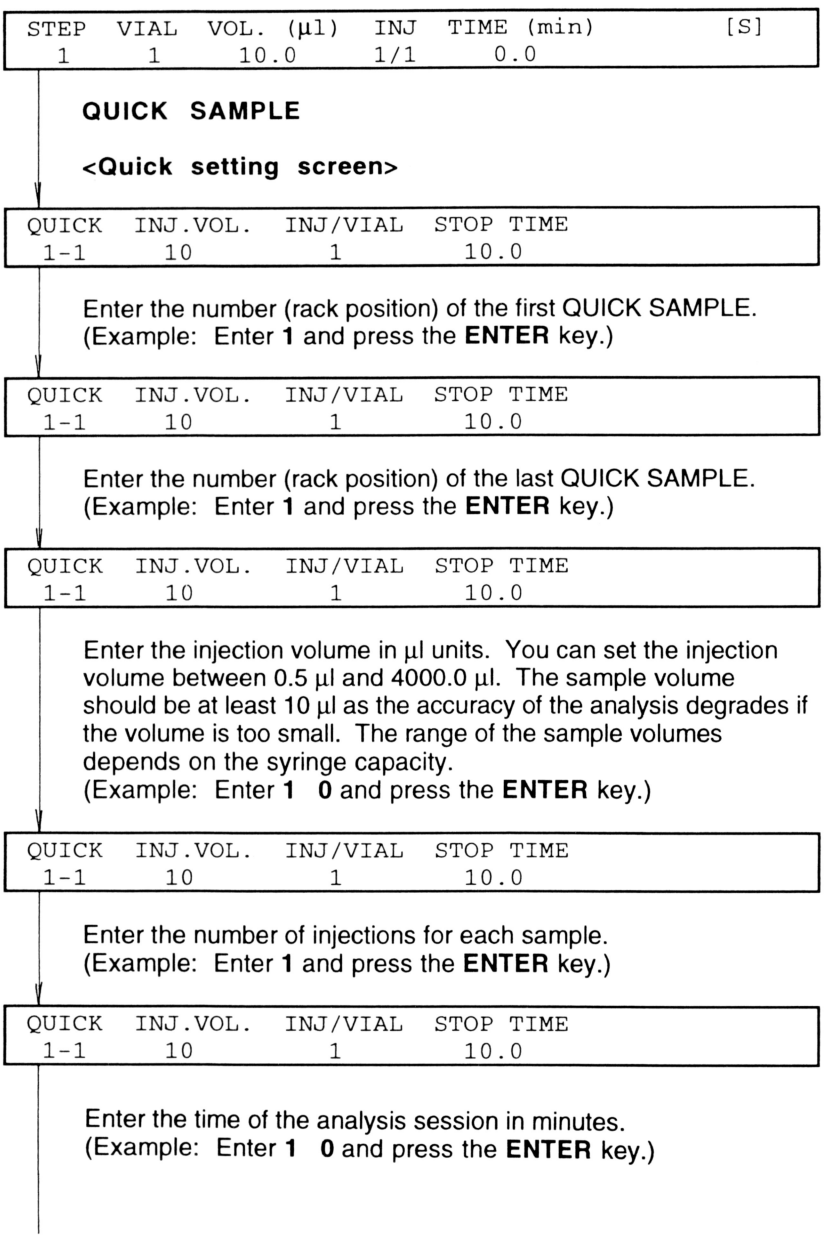
Figure 2-9 Logbook

2.3.3 Quick Sample Analysis

Press the **QUICK SAMPLE** function to establish parameters to analyze one or a few samples without the need to enter a complete set of operating parameters.

Note: In Quick Sample Mode, the autosampler will start as soon you establish the parameters. Check that the samples are in the correct position before entering the analysis conditions.

<Initial screen>



When the instrument is in the standby mode, it will start as soon as you complete entering the data settings (i.e. when you press **ENTER** after the STOP TIME data entry).

Notes:

1. You can use Quick Sample analysis while running an analysis or when the instrument is in the standby mode waiting to start.
2. When you specify Quick Sample analysis during execution of other analysis, the quick analysis starts upon completion of the current step in the operating analysis table.
3. When you execute Quick Sample analysis during an ordinary analysis session, the ordinary session resumes after the completion of the Quick Sample.
4. The operations defined by Quick Sample analysis will NOT take place if you press the ESCAPE key while entering the analysis conditions for the Quick Sample.
5. You can not use the Quick Sample analysis mode when the communication mode is set to the D-line mode. This is to prevent discrepancies between analysis conditions set via D-line communication and the Quick Sample analysis conditions.

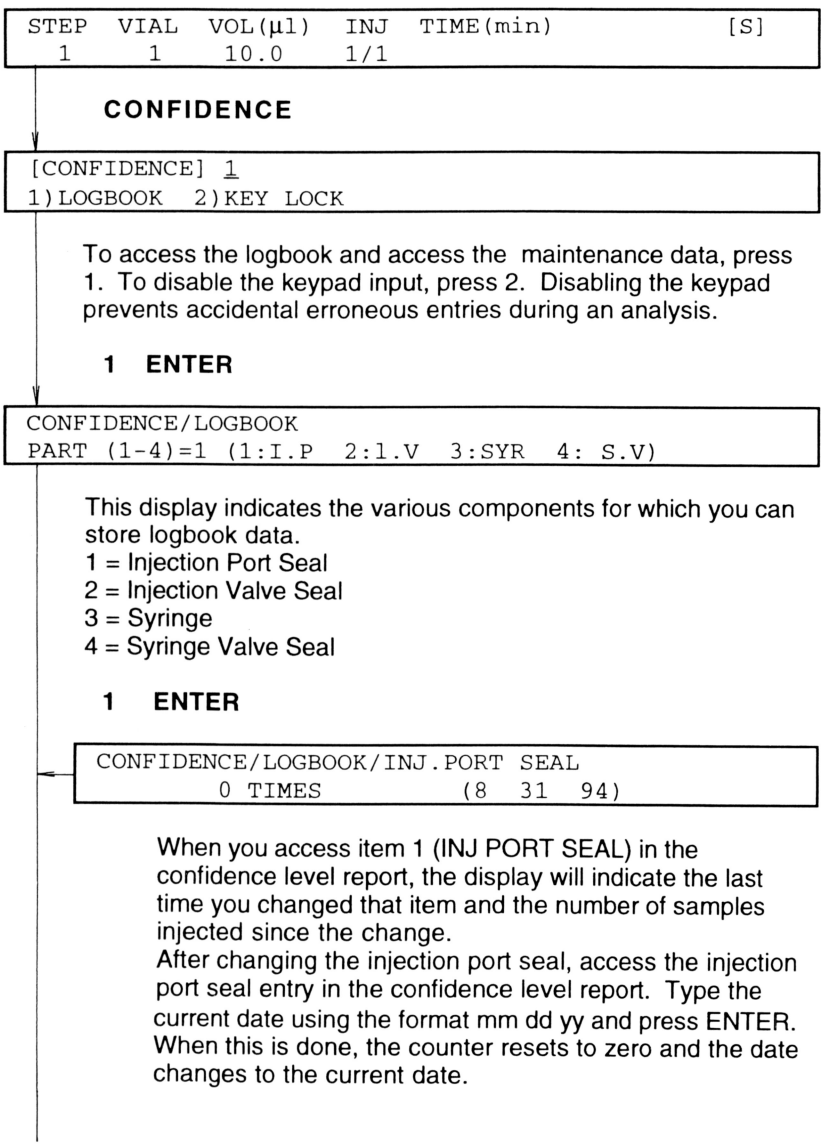
2.3.4 Confidence Level Report

This autosampler provides a Confidence Level Report function. This function gives:

- access to a logbook that gives the number of injections that have been made after a replaceable component was changed. The logbook allows to record the date of that change.
- a lockout function to restrict access to certain keyboard functions.

To use the features of the Confidence Level Report, press the **CONFIDENCE** key.

<Initial screen>



2 ENTER

```
CONFIDENCE/LOGBOOK/INJ.VALVE SEAL
        0 TIMES          (8  31  94)
```

When you access item 2 (INJ VALVE SEAL) in the confidence level report, the display indicates the last time that a change of the item took place and the number of samples injected since that change.

After a change of the injection valve seal occurs, access the injection port seal entry in the confidence level report. Type the present date in the format mm dd yy and press ENTER. At this point, the counter resets to zero and the date changes to the current date.

3 ENTER

```
CONFIDENCE/LOGBOOK/SYRINGE
        0 TIMES          (8  31  94)
```

When you access item 3 (SYRINGE) in the confidence level report, the display indicates the last time that a change of the item took place and the number of sample injections since that change.
After you change the syringe, access the syringe entry in the confidence level report. Type the present date in the format mm dd yy and press ENTER. When this is done, the counter resets to zero and the date changes to the current date.

4 ENTER

```
CONFIDENCE/LOGBOOK/SVALVE SEAL
        0 TIMES          (8  31  94)
```

When item 4 (SVALVE SEAL) in the confidence level report is accessed, the display indicates the last time that a change the item took place and the number of sample injections since that change.
After you change the syringe valve seal, access the syringe valve seal entry in the confidence level report. Type the present date in the format mm dd yy and press ENTER. When this is done, the counter resets to zero and the date changes to the current date.

To disable the ability to input data from the keypad, select 'KEY LOCK'.

```
[CONFIDENCE] 2
1)LOGBOOK  2)KEY LOCK
```

2 ENTER

```
CONFIDENCE/KEYLOCK
KEY LOCK (0-1)=0   (0: NO  1: YES)
```

Press 1 and then **ENTER** to set the key lock state. When the key lock is active, only the **ESC** key is functional. To cancel the key lock state, press the **ESC** key.

Notes:

1. The key lock state cancels when power is turned off and on again.
2. When you observe an error occurs while the keypad is locked, the keypad lock state ceases (i.e. the keys will become active).

2.4 Stop Procedures

1. When the analysis of the last specified sample in the rack concludes or when the vial detector senses the last vial during an analysis session, END displays on the screen and the buzzer sounds. Press any key to return to the initial screen.

2. ALL END displays on the screen and a buzzer sounds 15 minutes after END displays, if you do not press any key. The display will show the step number, the number of the last sample vial which was injected, and the injection number at the time the session ended. Press any key to return to the initial screen.

3. Use the **STOP** key to interrupt an analysis session.

4. Finally, stop the pump and the recorder.

IMPORTANT:

1. When you use a mobile phase that contains dissolved salts (e.g. buffers), take precautions to ensure that the dissolved salts do not precipitate in the system. The presence of solids may scratch the surface of the valve seal and/or clog up the flow path. After use, wash the mobile phase flow path thoroughly with distilled water before shutting down the system.
2. When you do not plan to use the instrument for a long time, clean the mobile phase flow path with a solvent that can dissolve any particulate matter that may be present in the instrument. After this cleaning procedure, pump distilled water through the system to displace any organic solvent that may be present.

2.5 Operational Precautions

2.5.1 Setting the Cut Volume and the Syringe Speed

Section 3.2.2 describes the methods this instrument uses to inject samples.

The Cut Volume method dilutes a sample while transferring it. As a result, the concentration will be less than when you use a manual injection valve. The degree of the dilution depends on the cut volume and syringe speed. Note the following:

1. If you do not need an absolute area (intensity) value (e.g. you analyze the standard in the same way as the samples), set the cut volume to about 20 µl and the syringe speed to "1". This will ensure good repeatability. The degree of dilution when you use methanol is 10%.

2. If you need an absolute area (intensity) value, set the cut volume to 50 µl or more and the syringe speed to "1". The sample dilution is a few percent.

3. The injection volume of an external standard sample should be the same as that of an unknown sample.

2.5.2 Injection Volume and Cut Volume

The size of the syringe and the dead volume of the system determine the maximum injection volume. Normally, you will use a 0.5 ml syringe in the sequential mode. The sample is taken up into the tube between the needle and the syringe.

The following expression defines the limits for the injection and the cut volumes:

vi + vl + vr <500 µl - 45.0 µl - 10.0 µl = 445 µl

Where

vi : Injection volume

vl : Cut volume (Lead volume)

vr : Cut volume (Rear volume)

45 µl : The dead volume of the tube between the injection port and the injection valve

10 µl : The volume of the air layer before and after the sample (5 µl × 2)

The dead volume of the tube between the injection port and the injection valve is 45.0 µl and the volume of the air layer before and after the sample is 5.0 µl × 2; the sum of the injection volume, the lead volume and the rear volume calculates to 445 µl when you use the 0.5 ml syringe.
The volume you inject will be smaller if the lead volume and/or rear volume is set to a non-zero value.
If the values for the injection volume or the cut volume are larger than that permitted by the above expression, an error message will display.

2.5.3 Precautions when Performing Injections Using the All Volume Injection Method

1. Maximum Injection Volume: The following expression shows how the injection volume depends on the feed volume when you employ the all volume injection method.
(When a 500 µl syringe is used)

$vi + vf < 500\ \mu l - 45.0\ \mu l - 10.0\ \mu l = 445\ \mu l$

vi : Injection volume
vf : Feed volume
45 µl : The dead volume of the tube between the injection port and the injection valve
10 µl : The volume of the air layer (5 µl × 2)

When you employ a 500 µl syringe, the dead volume of the tube between the injection port and the injection valve is 45.0 µl and the volume of the air layer before and after the sample is 10.0 µl (5 µl × 2); the sum of the injection volume and the feed volume calculates to 445 µl.

2. Injection Volume and Feed Volume: The feed volume is set so that the front end of the sample is normally at the center position of the sample loop when you inject the samples in the all volume injection method. The L-7250 provides a 100 µl sample loop as the standard; when you use a 10 µl sample, the feed volume is generally set at 30 to 40 µl.

2.5.4 Notes on Sample Injection with the Full Loop Method

1. Maximum Injection Volume: The following expression shows how the injection volume depends on the feed volume when you employ the full loop injection method. (Refer to Figure 3-4).

In case of a 500 µl syringe:

Vi + Vw < 500 µl - 45.0 µl - 10.0 µl= 445 µl

Where,

Vi : Injection volume

Vw : Waste volume

45 µl : Dead volume in tubing between injection port to injection valve

10 µl : Volume of air layer (5 µl × 2)

In the full loop sample injection with a 500 µl syringe, the dead volume of the tube between the injection port and the injection valve is 45.0 µl and the volume of the air layer before and after the sample is 10.0 µl (5.0 µl × 2). The sum of the injection volume and the waste volume calculates to 445 µl.

2. Injection Volume and Waste Volume. The first half of the waste volume occurs at the top of the sample you inject, and the second half at the end of the sample.

2.5.5 Eluent and Wash Solution

1. Use the same solvent for the eluent and the wash solution. When you use a gradient, the composition of the wash solution should be the same as the initial mobile phase composition.

2. Degas both the eluent and the wash solution.

3. Replace the wash solution periodically to prevent bacterial growth.

3 PRINCIPLES, FUNCTIONS AND SPECIFICATIONS

3.1 Application

This autosampler aids in automatic analyses when you use it as part of a liquid chromatography system.

3.2 Function

3.2.1 Configuration

Figure 3-1 shows the configuration of the L-7250 Autosampler. Samples to be analyzed are in a rack inside a chamber. The chamber also includes the sampling arm. The sampling arm has a syringe to allow aspiration of the samples. You can move the arm along the transverse axis (X), the longitudinal axis (Y), and the vertical axis (Z). The injection valve places the samples into the mobile phase flow path. You can choose syringes with a delivery volume of 0.1, 0.5 ml, 1 ml, 2.5 ml and 5 ml. You can set the analytical conditions via the keypad. The display panel presents information about the operational status of the system.

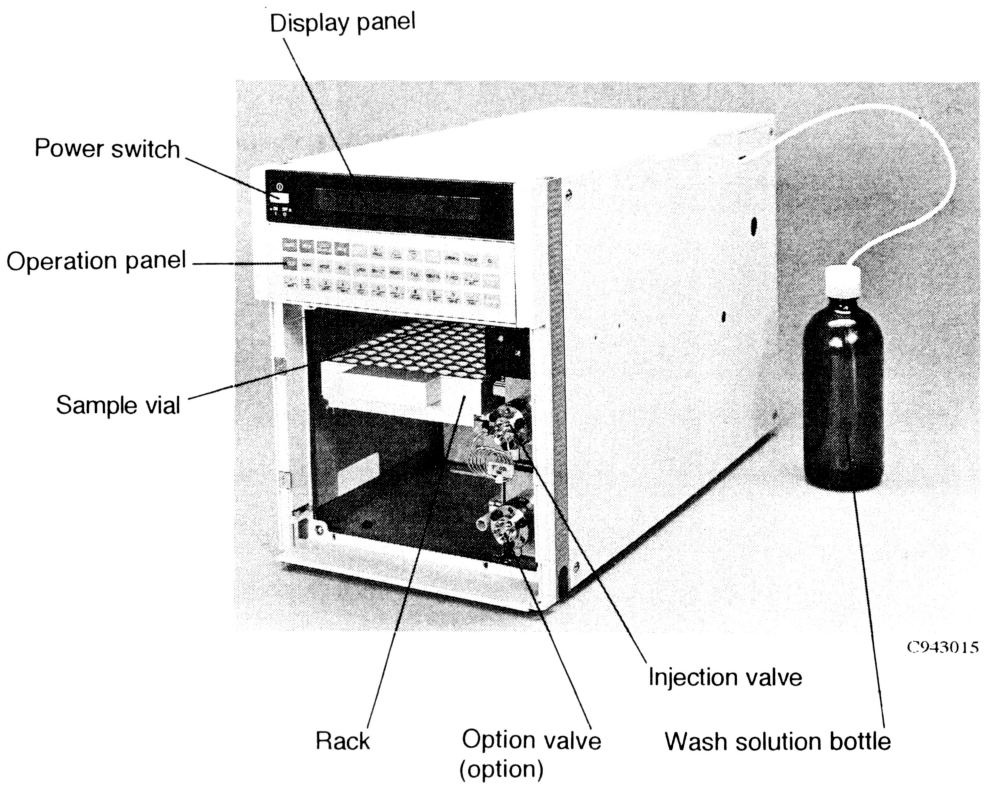


Figure 3-1 Configuration of the L-7250 Autosampler

3.2.2 Functions

The L-7250 has two operation modes to provide various functions. One mode is the sequential mode which analyzes the samples on the rack in sequence, and the other is the programming mode which permits sample preparation. Use the **Mode** key for mode selection.

3.2.3 Sample Injection Methods

The L-7250 permits three injection methods. In the standard injection method, the amount of sample that the needle withdraws is slightly larger than the amount of sample that the unit transfers from the injection valve to the column. The autosampler injects the middle part of the sample; the unit does not inject the leading and trailing ends of the sample. We refer to this method as the cut injection method.

Refer to Section 4.5.2 to 4.5.4 of the ADVANCED OPERATION manual for additional information about injection procedures.

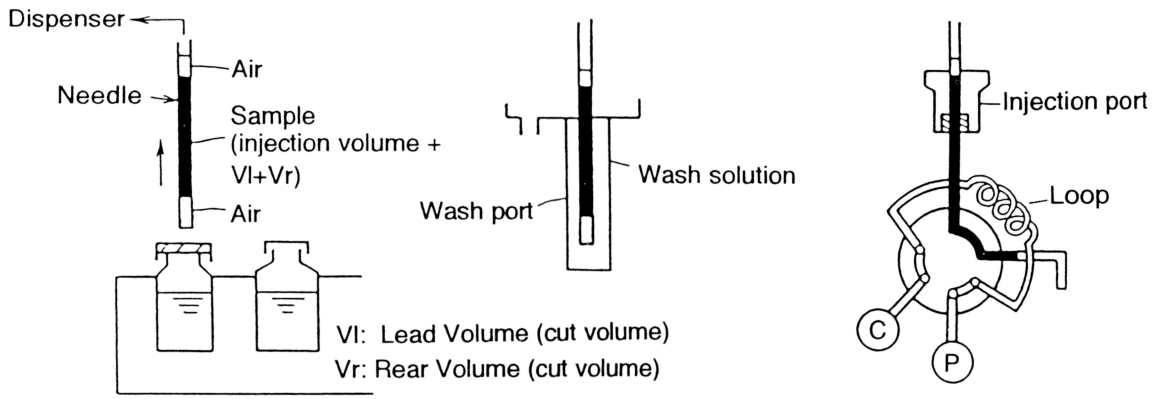
If the amount of a sample is small, the entire sample that the needle withdraws is transferred into the sample loop and into the column. We call this method the all volume injection method. In the full loop injection method, the sample loop fills completely with the sample for measurement.

1. Cut Injection Method Figure 3-2 shows the cut injection method. This method provides good reproducibility, but you will waste some of the sample.

(1) The syringe aspirates into the needle a sample that is "sandwiched" between two layers of air.

(2) The unit washes the outside of the needle.

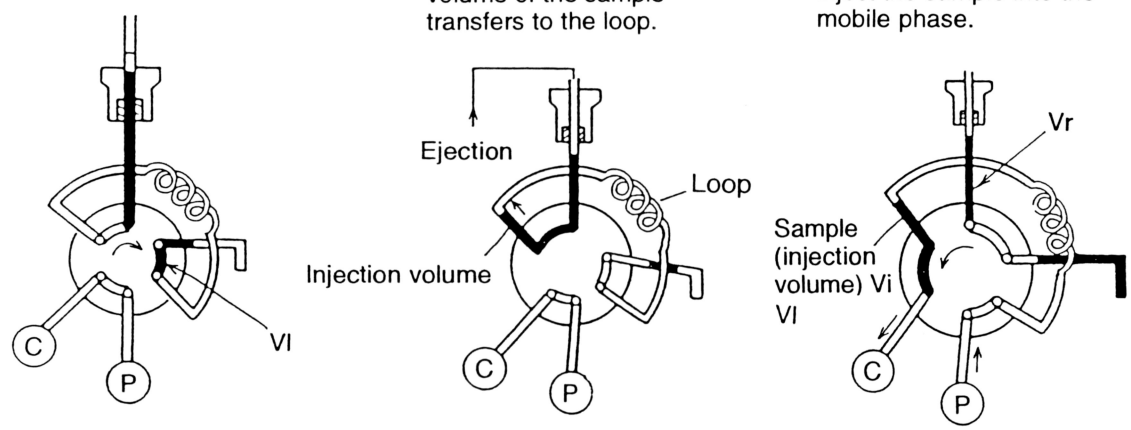
(3) The needle comes into the injection port and the sample is transferred to the valve.



(4) The flow path switches.

(5) The specified injection volume of the sample transfers to the loop.

(6) The flow path switches to inject the sample into the mobile phase.



Vl: Lead volume (cut volume)

Vi: Injection volume

Vr: Rear volume (cut volume)

(7) After injection, the instrument washes the paths inside the valve.

(8) The needle moves to the wash port and the wash solution cleans the needle.

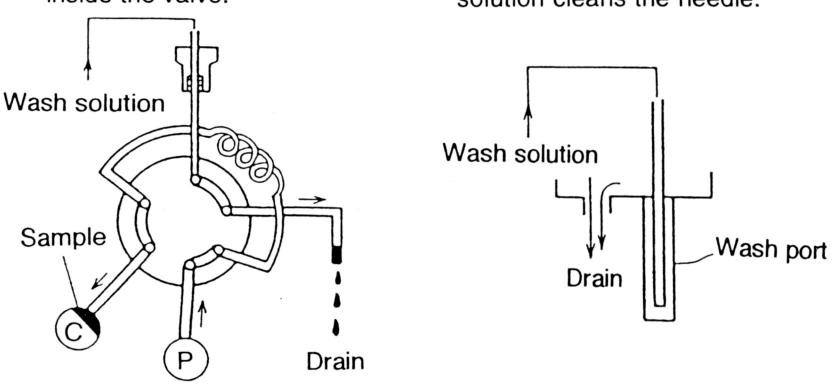


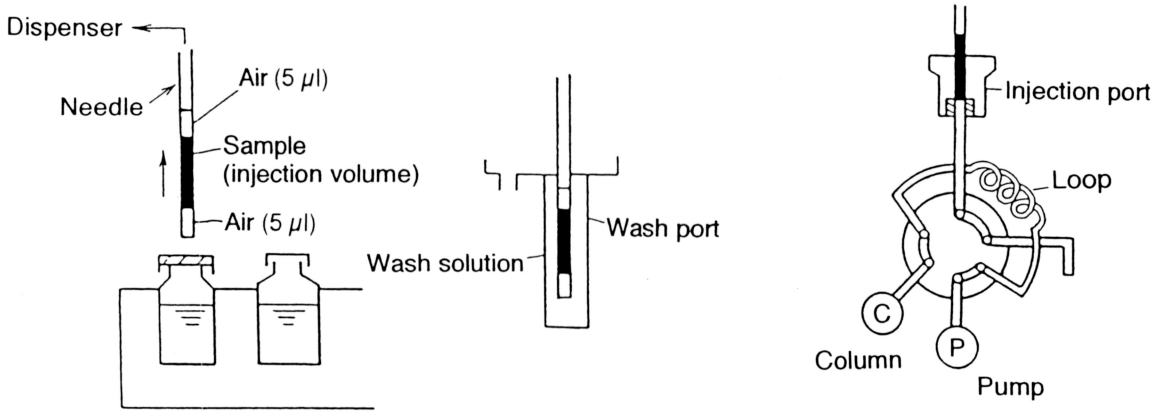
Figure 3-2 Cut Injection Method of a Sample

2. All Volume Injection Method Figure 3-3 shows the all volume injection method. This method is suitable for analysis of minute samples since there is no sample waste.

(1) The syringe aspirates into the needle a sample that is "sandwiched" between two layers of air.

(2) The unit washes the outside of the needle.

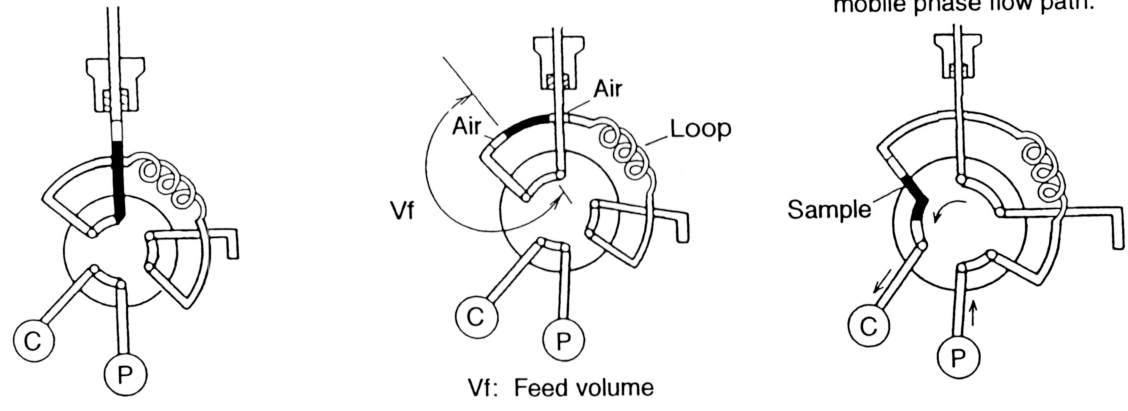
(3) The needle inserts into the injection port.



(4) The flow path switches.

(5) The sample transfers to the loop.

(6) The flow path switches to inject the sample into the mobile phase flow path.



Vf: Feed volume

(7) After injection, the instrument washes the sample flow paths.

(8) The needle moves to the wash port and the wash solution cleans the needle.

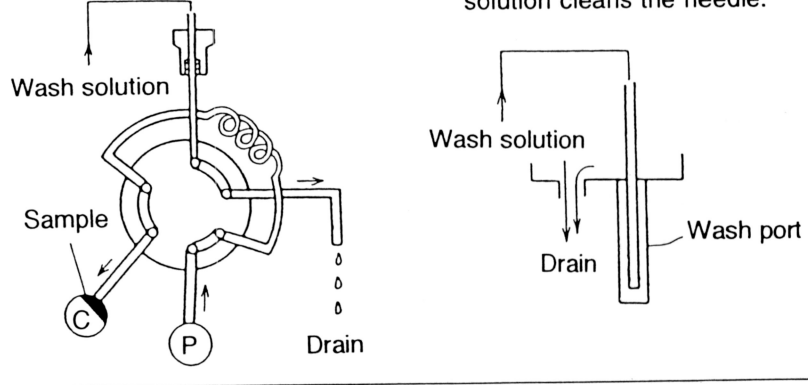
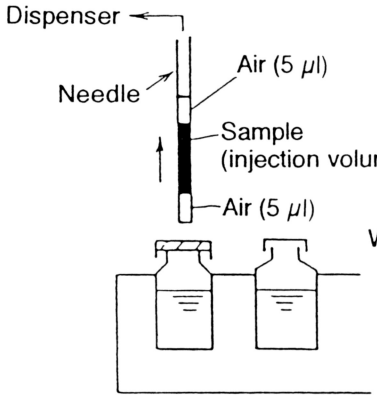


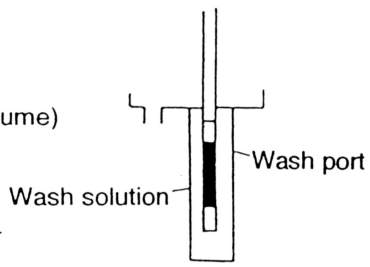
Figure 3-3 All Volume Injection Method of a Sample

3. Full Loop Injection Method Figure 3-4 shows the full loop injection method. This method provides the best repeatability of sample injection volume in an analysis.

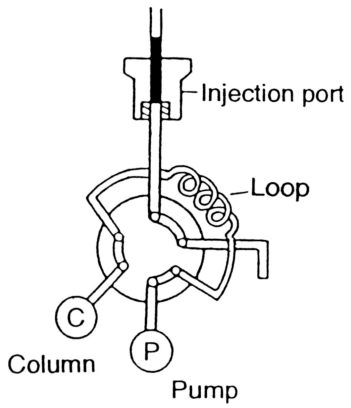
(1) The syringe aspirates into the needle a sample that is "sandwiched" between two layers of air.



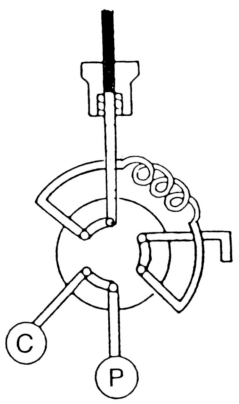
(2) The unit washes the outside of the needle.



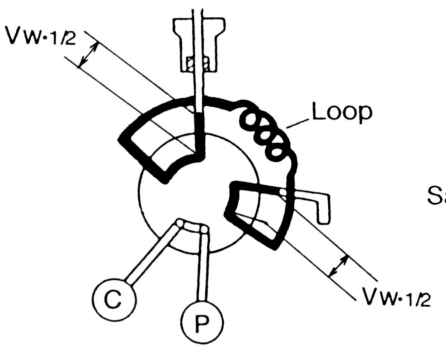
(3) The needle is placed in the injection port.



(4) The flow path switches.

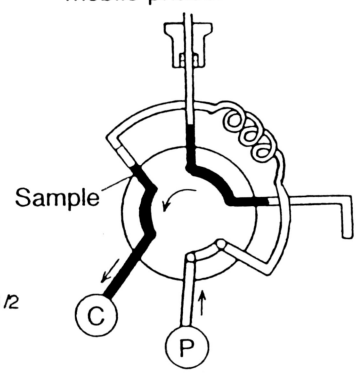


(5) The sample transfers to the loop.

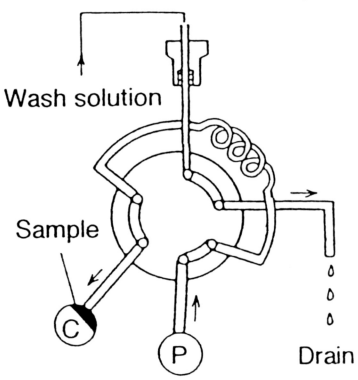


Vw : Waste Volume

(6) The flow path switches to inject the sample into the mobile phase.



(7) After injection, the instrument washes the valve flow paths.



(8) The needle moves to the wash port and the wash solution cleans the needle.

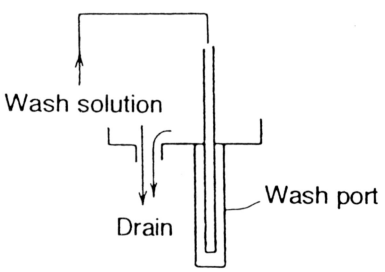


Figure 3-4 Full Loop Injection Method of a Sample

3.3 Specifications

(1) Number of standard samples: 120

(2) Rack/sample vials:
Conditions can accommodate a variety of sample vials and racks

(3) Syringe capacity: 0.5 ml (standard syringe)
0.1, 1.0, 2.5, 5 ml (optional)

(4) Sample injection volume:
0.5 µl to 400 µl (standard loop: 100 µl)

(5) Syringe speed: the analyst can choose among 5 speeds

(6) Sample injection method:
Syringe measurement cut injection
Syringe measurement all volume injection
Full loop measurement injection

(7) Pressure resistance: 34 MPa

(8) Wetted-part materials:
Stainless steel (SUS316), tetrafluoroethylene (Teflon), borosilicated glass, Vespel*, PEEK** (polyethyl ether ketone), EPDM***, polypropylene.

* Vespel : Not applicable to a pH other than 1 to 13.

** PEEK : Not applicable to concentrated sulfuric acid, tetrahydrofuran (THF) and methylene chloride.

*** EPDM : Not applicable to benzene, hexane, toluene, xylene, chloroform, tetrahydrofuran, hydrochloric acid, nitric acid, etc.

(9) Display: LCD with back light (40 characters × 2 lines)

(10) Control system:
- Sequential injection function for LC
- User Programmable (Sample preparation function)
- External computer control via RS-232C

(11) Vial detecting function: Provided

(12) Data storage: Battery backed-up memory

(13) Number of injections per sample: Unlimited (Up to 99 times in the sequential mode)

(14) Analysis time: Unlimited (999.9 minutes in the sequential mode)

(15) External communication function: D-line communication

(16) GLP function:

Error detection : Detects abnormality in operation of the system.

Maintenance logbook: After part replacement, records the number of injections. The count can be reset to zero after replacement of certain parts (e.g. injection port seal).

(17) Ambient temperature : 4 to 35° C

(18) Ambient humidity : 45 to 85%

(19) Power requirement : 100 - 240 V AC ±10%, 50/60 Hz

(20) Installation category : II in IEC1010-1 Annex J

(21) Power consumption : Approx. 85 VA

(22) Dimensions : 260(W) × 500(D) × 320(H) mm (excluding protrusions)

(23) Weight : Approx. 25 kg

4 INSTALLATION

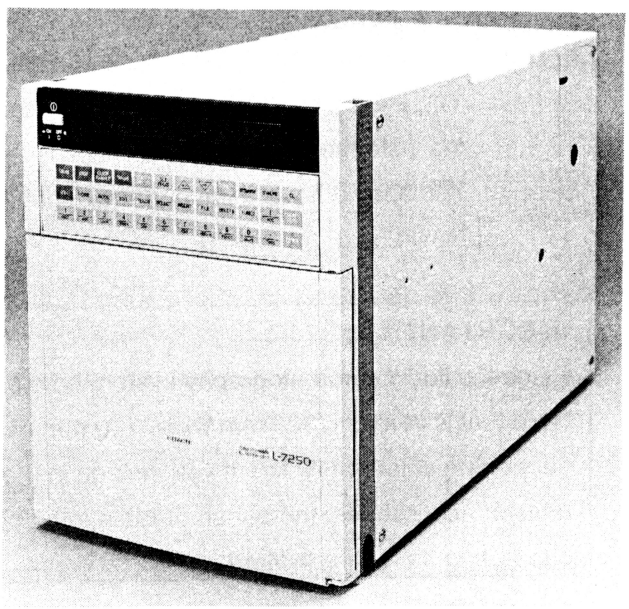
4.1 Unpacking

⚠ CAUTION

Heavy Instrument!

This instrument weighs as much as 25 kg. Dropping it when carrying could result in serious injury. Firmly hold the front and rear parts of the instrument when carrying.

Carefully remove the autosampler from its shipping box.



C943014

Figure 4-1 L-7250 Autosampler

4.2 Checking Contents in Package

After unpacking, check the contents against the packing list. If any item is missing or damaged, contact your dealer immediately.

4.3 Installation Requirements

4.3.1 Electrical Power and Space Requirements

Before installation, check the following conditions.

1. Power Source

Voltage : 100 - 240 V

The line voltage must be within ±10% of the rated voltage. You do not need to select the voltage.

Frequency : 50 or 60 Hz
The frequency must be within ±4% of rated frequency. You do not need to select the frequency.

Capacity : 85 VA or more
The power source should provide sufficient power for all components of the system (e.g. pump, detector, integrator, etc.).

Ground line : Ground resistance 100 ohms maximum

2. Space Requirements

Bench : 350 mm wide and 700 mm deep (minimum).
You should provide a space of at least 200 mm behind the instrument.

Floor : You should have a flat surface capable of supporting at least 25 kg.

IMPORTANT:
Place the liquid chromatography system on a bench or laboratory table that is at least 1300 mm wide, 700 mm deep and capable of supporting at least 170 kg. If you include in the system additional units or accessories, the bench or table must be capable of supporting the entire system.

4.3.2 Environmental Considerations

1. Operating Temperature: 4 to 35°C
Install the system in a room in which the temperature is maintained at 20 to 25°C.

2. Operating Humidity: 45 to 85%

3. Laboratory Atmosphere:

3a. must have proper ventilation.

3b. must be free from acidic or alkaline gases which may corrode metals.

3c. must be free from organic solvent vapors (e.g. benzene, thinner) which may dissolve paint.

4. Other Precautions:

4a. Do not place the instrument in a location where the temperature undergoes significant change (e.g. under an air conditioning duct or by a window). Significant changes in temperature affect the performance of the unit.

4b. Do not place the instrument in direct sunlight. Direct sunlight may create significant temperature changes which can affect the performance of the system. In addition, it will affect the exterior appearance of the system.

4c. Do not place the instrument in a location which is susceptible to physical shocks or strong vibrations. A physical shock or vibration may affect the alignment of various components in the system.

4d. Do not place the instrument near gas burners, electric heaters or ovens. The cover should never be exposed to temperature exceeding 70°C.

4e. Do not place the instrument near a device that generates a powerful electrical field (e.g. an electric welder, high-frequency electric furnace or transformer).

4f. Do not locate the instrument in an excessively dusty or dirty location. Maintain a clean laboratory to insure maximum performance.

4g. Do not place the instrument in a location where the line voltage fluctuates excessively. Line voltage fluctuations will lead to a noisy chromatogram.

4h. Do not connect the autosampler to a line with electrical devices that do not have noise suppressors (e.g. stirrers, vibrators). Minimize the frequency of power surges from such devices. Power surges from these devices most commonly occur when they are turned on or off.

IMPORTANT:
The autosampler includes a number of integrated circuits. These circuits may be damaged if you expose them to excessive line voltage fluctuations and/or power surges.

4.3.3 Items to be Provided by User

The user needs to provide the following items.

(1) Sample vials and rack which meet Hitachi specifications. Contact your sales office for information.

(2) Two waste bottles large enough to hold the detector waste and the autosampler waste (Do not combine the waste in a single bottle.)

(3) Two 500 ml bottles of HPLC grade methanol (Used for function tests made after installation.)

4.4 Assembly

4.4.1 Layout

Figure 4-2 shows the layout of the autosampler and the peripheral equipment. When you connect other units, make sure that the following lengths of tubings are as short as possible to minimize band broadening.

- tubing between the autosampler and the column
- tubing between the pump and the autosampler
- tubing between the column and the detector.

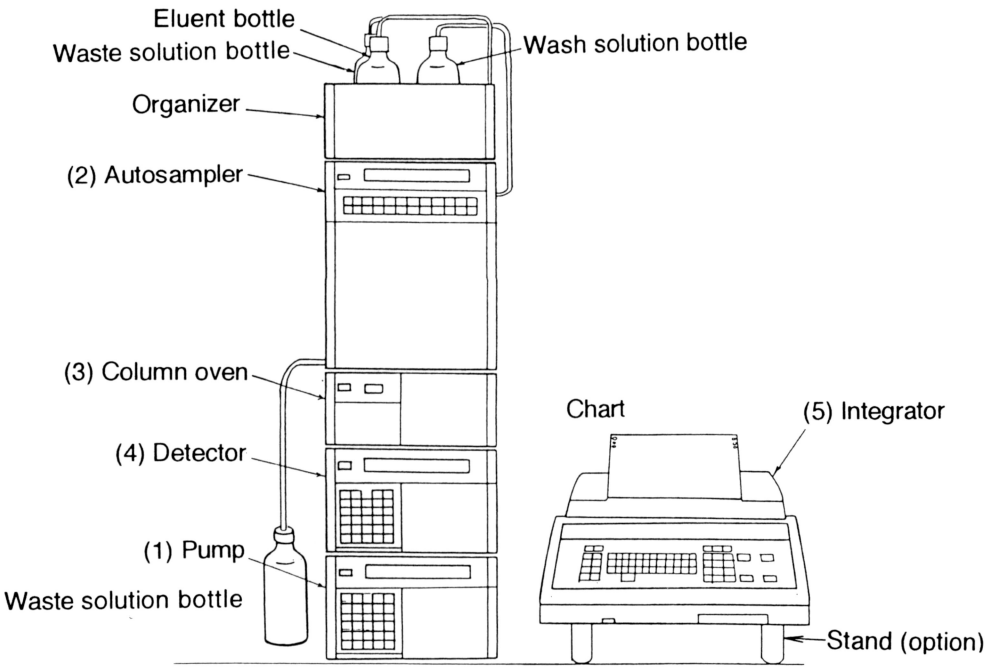


Figure 4-2 Typical Layout of Autosampler and HPLC System

4.4.2 Assembly

1. Removing the Strap and Packing from the Syringe A fastening strap and a packing protect the syringe from damage during shipment. You can access this strap and the packing by removing the side cover as shown in Figure 4-3. Remove the strap and the packing before using the instrument.

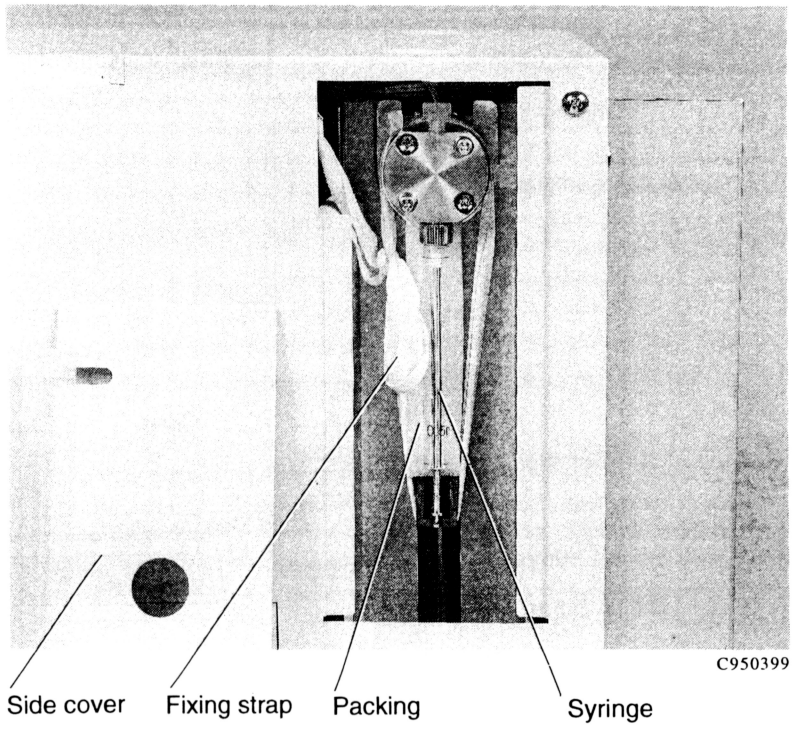


Figure 4-3 Removing the Fixing Strap from the Syringe

2. Removing the Transportation Fixture

2a. Loosen the X-axis arm screw. A screw protects the X axis arm during shipping. Loosen the screw as shown below before using the autosampler.

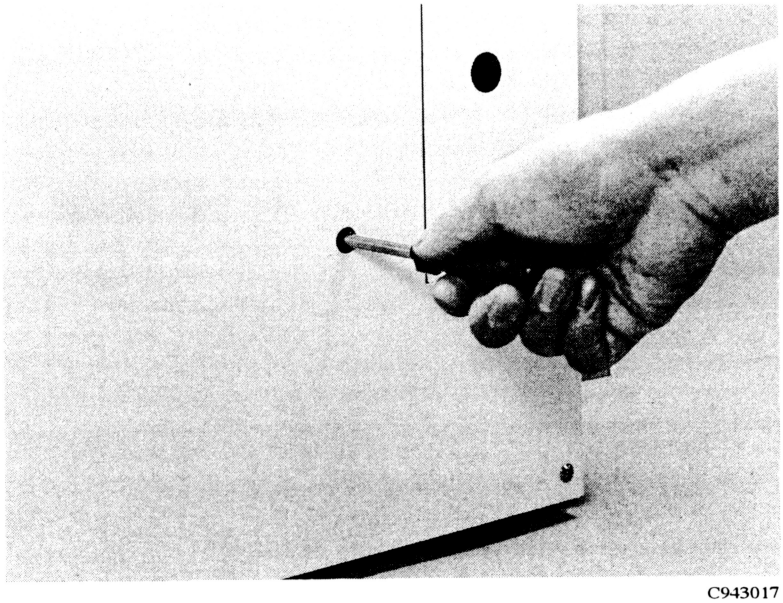


Figure 4-4 Loosening the X Axis Arm Screw

2b. A fixing strap and tape protects the arm during shipping. Remove the strap and the tape as described below.

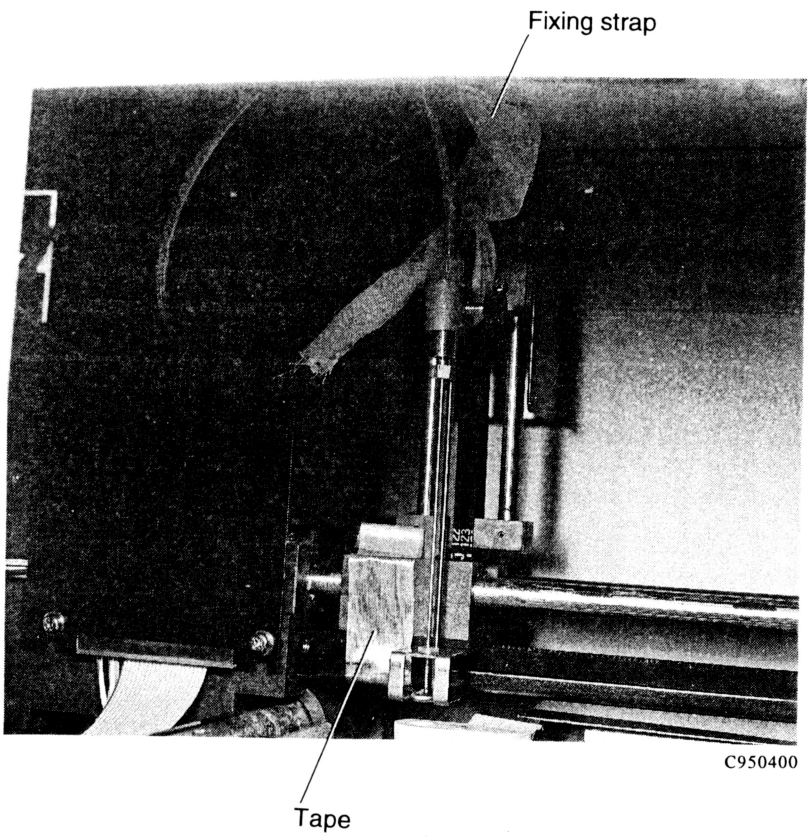


Figure 4-5 Removing the Strap and the Tape

4.4.3 Tubing Connections

1. Connect the injection valve of the autosampler to the pump and column.

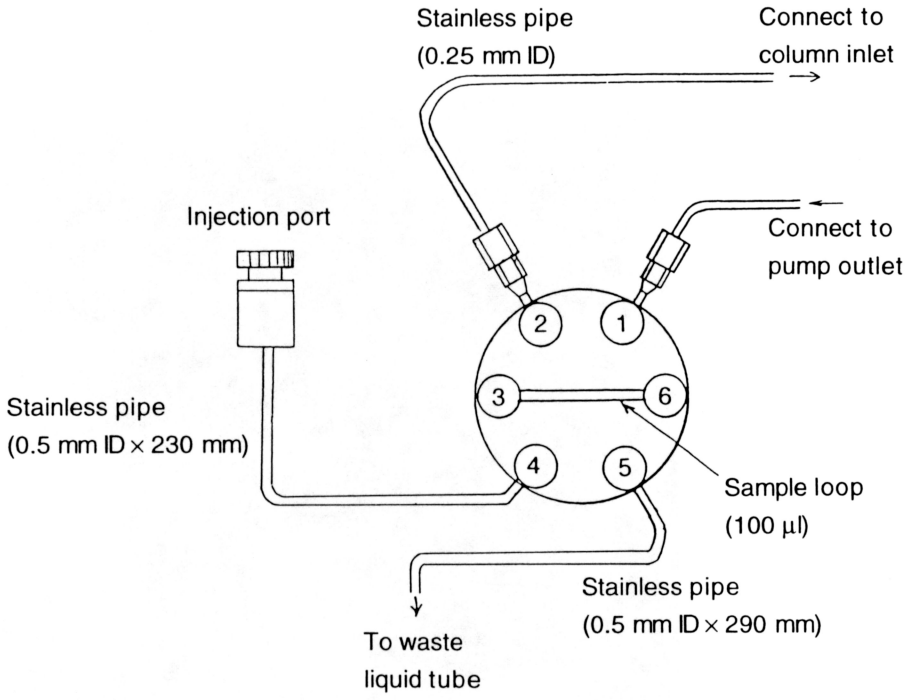


Figure 4-6 Injection Valve Tubing

IMPORTANT:

1. Make sure that the connecting tubing to the pump and the column is correct to ensure proper operation.
2. Connect the column after connecting the pump and after filling the tubing with the eluent from the injection valve to the column. If you do not fill the tubing with eluent, air may enter the column, and damage it.

2. Waste Tubing Insert the waste tubing in the waste bottle. Make sure that the bottle is large enough to hold the eluent.

IMPORTANT:

1. Place the waste bottle at a level lower than the autosampler. If the waste bottle is higher than the waste line of the autosampler, the waste solution will not enter the waste bottle.
2. The connection tubing between injection port and injection valve should be 0.5 mm in diameter and 230 mm in length. The tubing from the injection valve to waste bottle should be 0.5 mm in diameter and 290 mm long. If the size of tubing differs from the above dimensions, the performance of the instrument degrades.

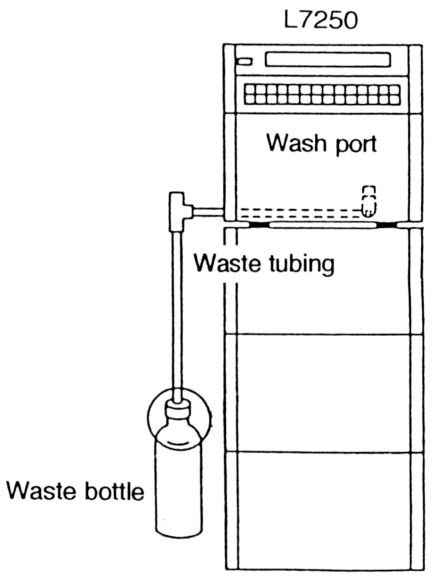


Figure 4-7 Waste Solution Tubing

IMPORTANT:

The waste tubing is made of chemical-resistant material. Note, however, that you can not use all kinds of solvents. For example, you can not use the following common solvents:

Benzene, hexane, toluene, xylene, chloroform, tetrahydrofuran (THF), hydrochloric acid, nitric acid, and other acids

4.4.4 Wiring

1. Connecting the Power Cord and the Ground Wire

> **⚠ WARNING**
>
> **Ground Properly to Prevent Electric Shock Hazard!**
>
> - Be sure to use the power cable supplied with the instrument. Use of a different power cable may result in an electric shock hazard.
> - This instrument is classified as "1" in IEC1010-1 Annex H and "plug-connected type" in IEC1010-1, so connect the power cable to a grounded 3-wire outlet.
> - If a grounded 3-wire outlet is not available, then be sure to provide proper grounding connection as shown in Figure 4-8.

1a. Plug the power cord into the power supply.

1b. When using an adapter plug, connect the ground wire securely to a true ground terminal.

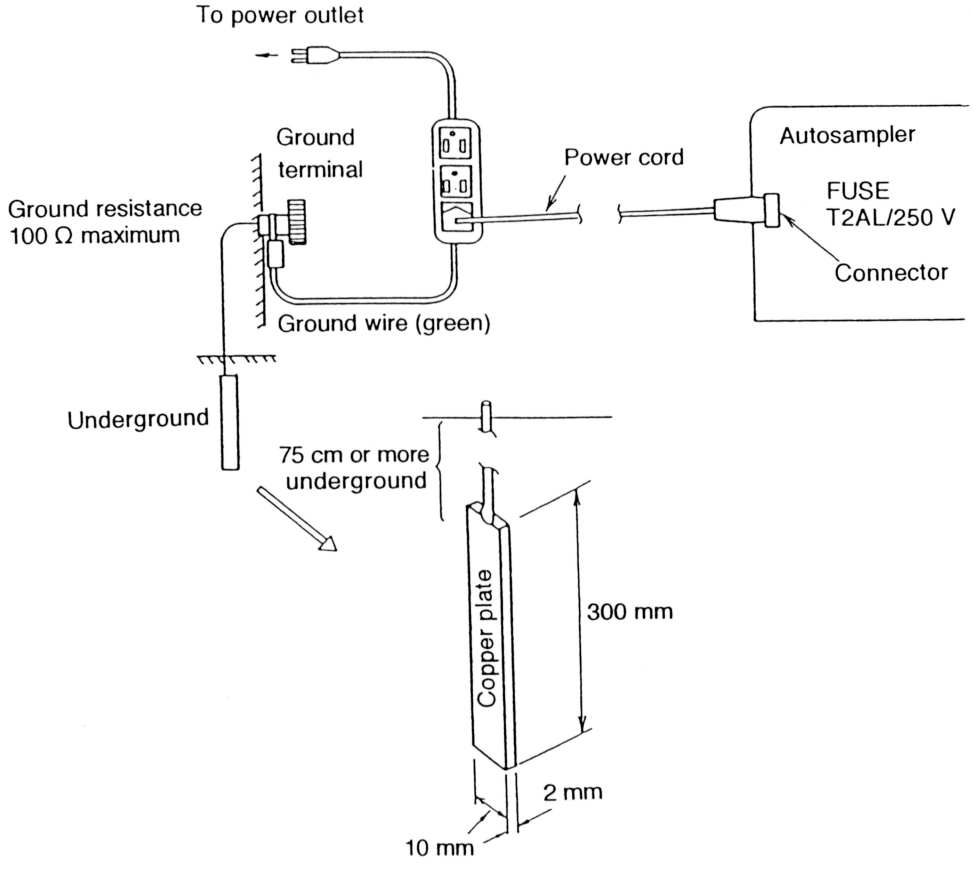


Figure 4-8 Grounding

2. Signal Line Connections

2a. D-line Connection The Hitachi analytical network "D-line" simplifies the connection of the various components of the system. Figure 4-9 shows the connections for a complete HPLC system. Refer to Section 5.1 "Communication Mode Setup" for information about setting the autosampler to work with the D-line communication program. ( ): For connection with the instrument bearing the CE conformity marking.

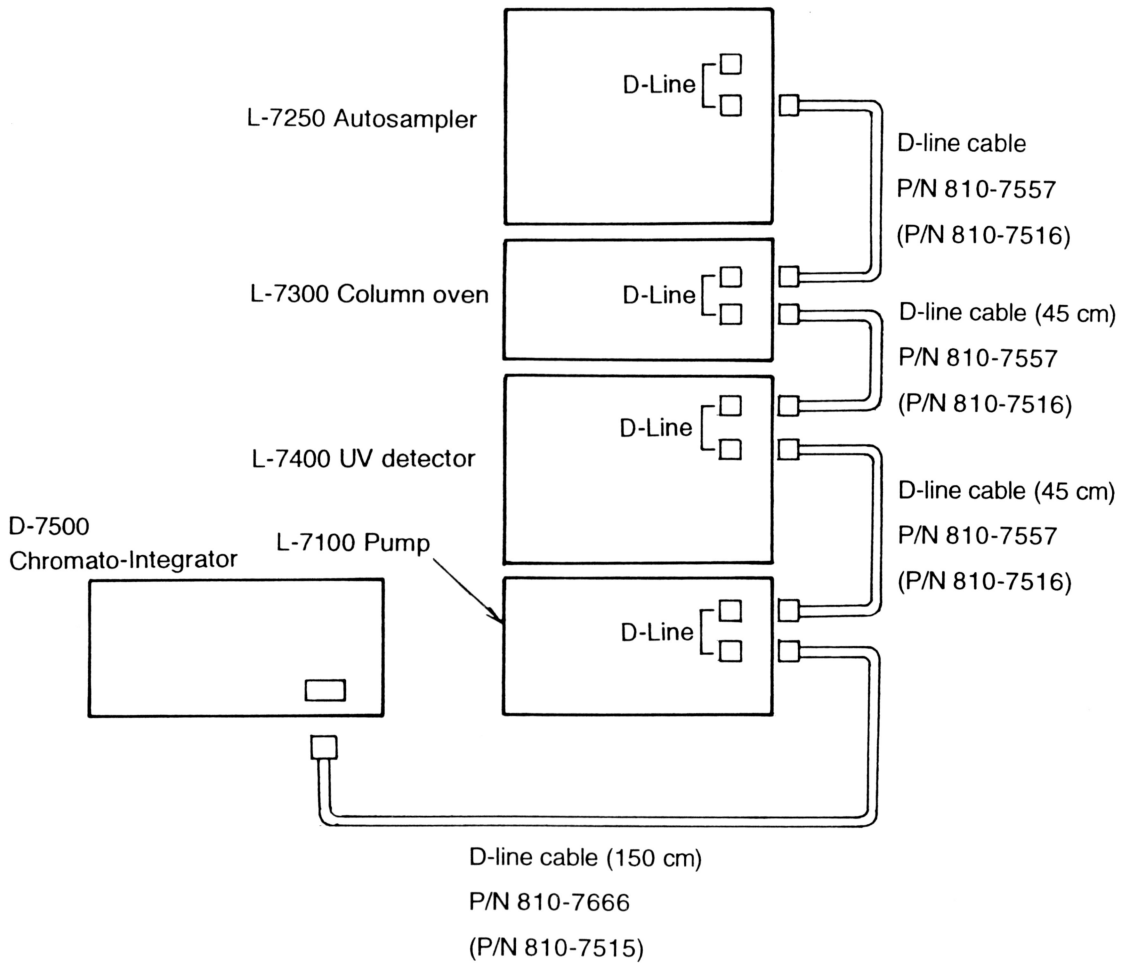


Figure 4-9 D-Line Cable Connections

2b. Connection with the D-2500 Integrator Figure 4-10 shows the simplest signal line connection scheme for using the Model L-7250 Autosampler in combination with the D-2500 Integrator. Data processing by the D-2500 starts when the unit receives the end-of-injection signal from the L-7250 autosampler.
( ): For connection with the instrument bearing the CE conformity marking.

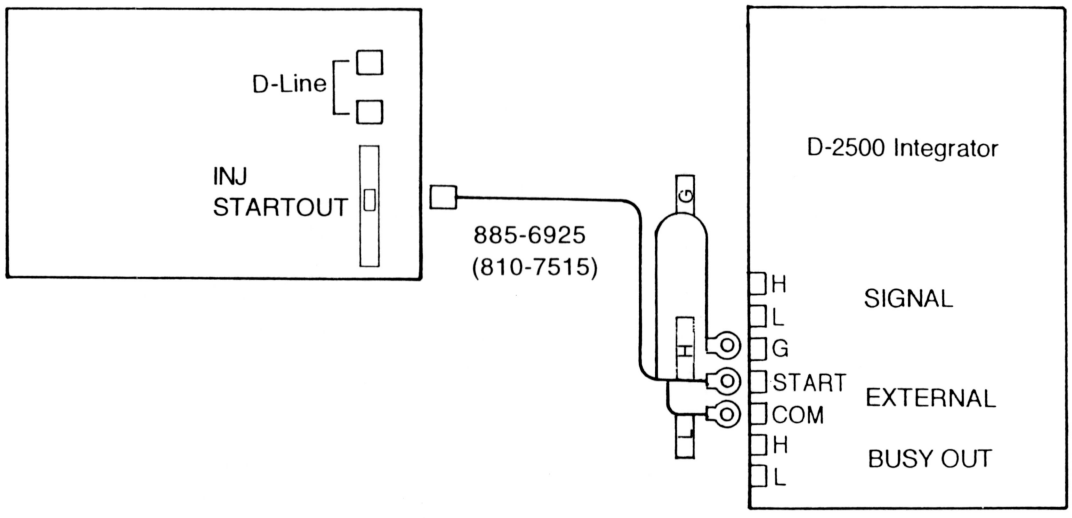


Figure 4-10 Connection with D-2500 Integrator

2c. Connection with Other Instruments To connect the autosampler to any other Hitachi data processor or to a system from another manufacturer, use the relay box (P/N 810-7630). This box, which is available as an accessory, should connect as shown in Figure 4-11. ( ): For connection with the instrument bearing the CE conformity marking.

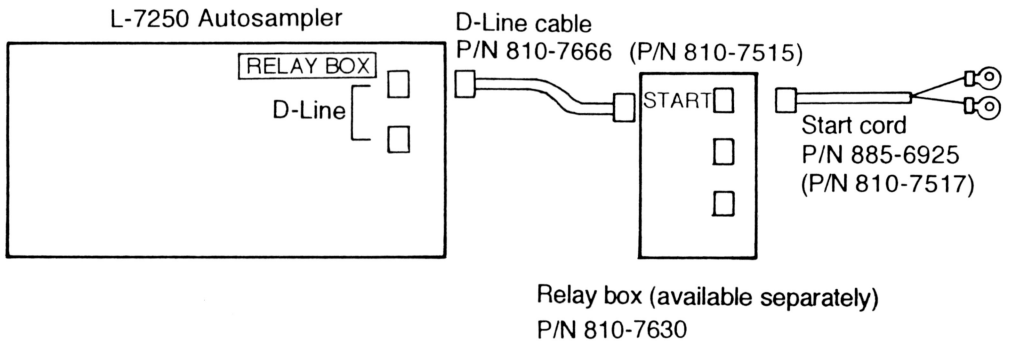


Figure 4-11 Relay Box Connection

Note: The relay element in the relay box has a maximum contact current capacity of 0.5 A and a power rating of 10 W.

2d. Contact Signal Input In addition to the D-line communication system, you can control the Model L-7250 autosampler with the external contact signals (See Table 4-1.) Figure 4-12 shows the contact input circuit scheme.

Table 4-1 Contact Signal Input

No.	Designation	Function	Signal
1	SERIES START IN	Serves as the external start signal. This is functionally equivalent to the **START** key.	OPEN CLOSE 100 ms or more
2	A/S STOP IN	Serves as the external stop signal . Including an error signal. This is functionally equivalent to the **STOP** key.	OPEN CLOSE 100 ms or more

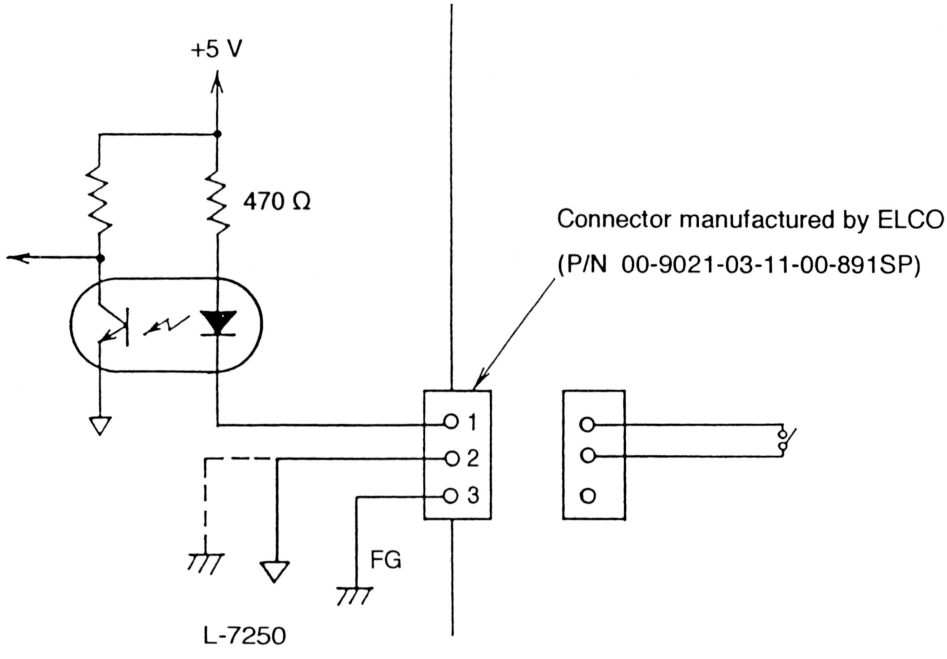


Figure 4-12 Contact Input Circuit

4.5 Operation Check

After you install the L-7250 autosampler, do the following:

- Pump eluent to the injection valve.
- Once the eluent fills the injection valve, turn on the power switch of the autosampler. When the initialization ends, the following initial screen will appear.

STEP	VIAL	VOL(µl)	INJ	TIME (min)	(S)
1	1	10.0	1/1	0.0	

- Check the operation of each key.

4.6 Performance Check

Section 6.2 gives a procedure for checking injection reproducibility.

5 MAINTENANCE AND PERIODIC CHECKING OF THE SYSTEM

5.1 Periodic Maintenance

5.1.1 Washing the Flow Path

Wash the flow path on a periodic basis. Bubbles will form if there is contamination in the flow path between the syringe and the needle; these bubbles will adversely affect the reproducibility of the system.

1. When Using an Aqueous Washing Liquid At the end of an analysis session, wash the entire system with distilled water. If the contamination is excessive, fill the syringe with detergent and leave it overnight. After washing the syringe, rinse it well with distilled water. If you do not plan to use the instrument for a week or longer, wash the flow path with distilled water and fill the syringe with distilled water. When the unit is left idle, you must fill the flow path with liquid. Do not allow the flow path to dry out. If the flow path dries out, trace contaminants in the washing liquid will deposit on the walls of the tubing and decontamination becomes very difficult.

Table 5-1 Recommended Neutral Detergents

Part No.	Detergent (Brand)	Concentration	Precaution
S264000	Extran	20 to 50 times	When using phosphorus-free neutral detergent (Extran MA03, etc.) dilute the detergent with water purified by an ion exchange or distilled water.
775-1365	Rinsing liquid	Not diluted	Fully remove the detergent from the system using the rinsing solution (or water of the same quality as indicated above) Prior to using the System again.

2. When Using a Mobile Phase Containing Salts or Buffers

When you use a mobile phase that contains salts, buffers, etc., it is possible that the buffer or salt may precipitate. This may cause clogging of the flow path and/or scratching of the valve seal. After using the system, wash the mobile phase flow path with distilled water. If contamination is excessive, fill the syringe with detergent and leave it overnight. After washing the syringe, make certain that you rinse it well with distilled water. If you plan not to use the instrument for a week or longer, wash the flow path with distilled water and fill the syringe with distilled water.

When the unit is left idle, fill the mobile phase flow path with liquid. Do not allow the flow path to dry out. If the flow path dries out, trace contaminants in the washing liquid will deposit on the walls of the tubing and decontamination becomes very difficult.

3. When Using Organic Solvents When you do not plan to use the instrument for some time, pump a clean organic solvent that is capable of dissolving all samples you injected into the system. Pumping a clean organic solvent will flush the flow path.

5.1.2 Cleaning

1. Clean the Wash Port Periodically If contamination of the wash port occurs, reproducibility of analytical results degrades and/or carry-over increases.

2. Clean the Wash Liquid Filter Periodically If the filter clogs, the washing fluid will not flow and solid materials will eventually deposit in critical areas. Solid material deposits lead to the premature wearing of critical components of the system.

5.1.3 Waste Liquid Tube

The waste tubes in the system are made from materials which have high chemical resistance (EPDM). These tubes will, however, tend to degrade over time when you use solvents such as benzene, hexane, toluene, chloroform, tetrahydrofuran (THF), and acids. If you need to use any of the above solvents, periodically check the tubing and the fittings. If you do not plan to use the unit for a period of time, rinse the flow path with an inert solvent that is miscible with the mobile phase to remove potentially corrosive solvents.

5.2 Checking the Performance and Specifications

5.2.1 Checking the Reproducibility

Check the reproducibility of the injection volume via the following procedure.

1. Items to be Prepared. You will need the following items for a performance check.

1a. Component units of liquid chromatography system:

- Pump (Hitachi L-7100 or equivalent)
- Detector (Hitachi L-7400 or equivalent)
- Chromato-data processor (Hitachi D-7500 or equivalent)
- Waste liquid bottle

1b. Parts for the autosampler reproducibility check

- Load resistance coil for reproducibility check - Use a coiled Teflon tube with an inner diameter of 0.25 mm and a length of 10 m.
- Methanol (HPLC grade) 500 ml × 2 pcs.
- Standard sample.

⚠ CAUTION

Perylene is mutagenic and carcinogenic. Use appropriate safety measures (gloves, fume hood, etc.).

- Sample vial, cap and septum
- Standard rack

2. Preparing the System for Operation

2a. Figure 5-1 shows how to connect the units for a performance check. Before powering up the system, check the tubing connections between the autosampler, pump, and detector.

2b. Connect the D-lines of the integrator and modules other than the autosampler. Disconnect the D-line of the autosampler. Change the communication mode setting of the autosampler to the D-line OFF mode. (See Section 2.1 in the L-7250 Advanced Operation Manual.)

Note: This setting is arranged in order to record the 10 injection response as a chromatogram in the format given in Figure 2 of the SOP in Section A.3. If the setting is not as above, then the data will be recorded separately at each 10 injection.

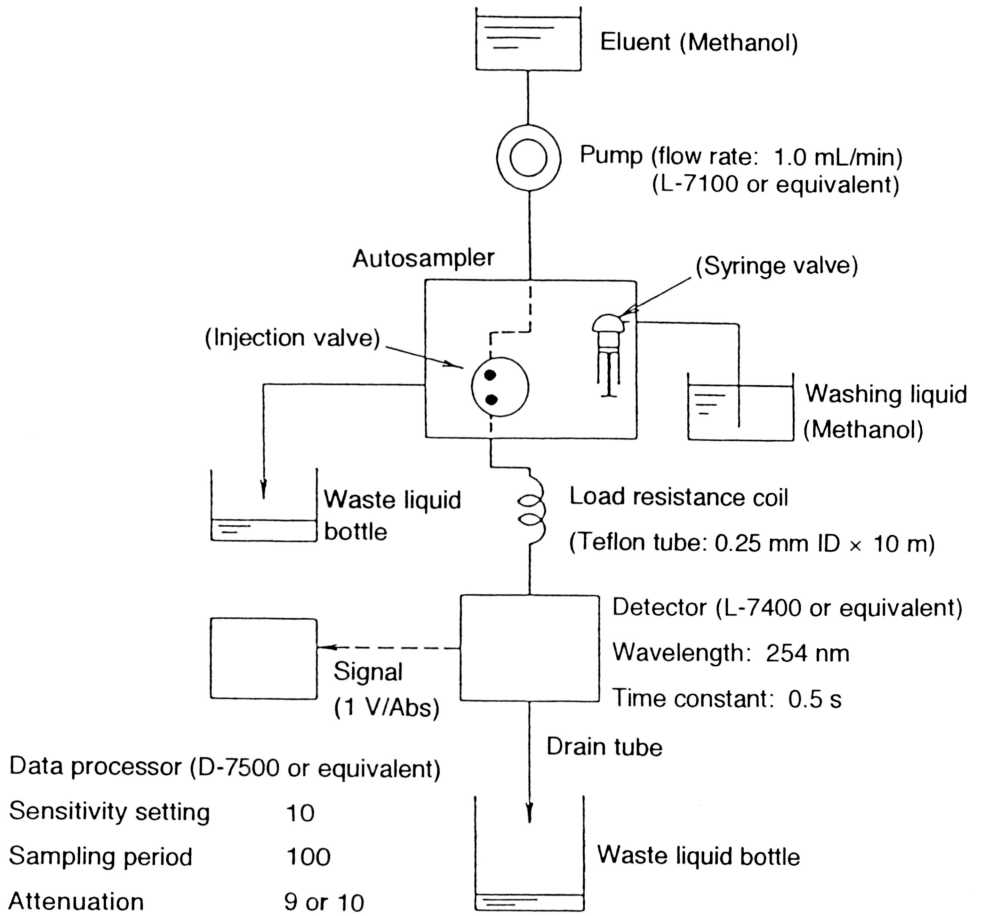


Figure 5-1 Checking the Performance of the System

2c. Check the drain tube connections to the autosampler waste liquid bottle. Position the waste liquid bottle lower than the autosampler.

2d. Follow the instructions in the respective instruction manuals to prepare all other units (e.g. pump, detector, etc.).

2e. Place the drain tube from the detector into the detector waste liquid bottle. Do not place it into the waste liquid bottle for the Model L-7250 autosampler. If you use a common waste bottle, you will observe a noisy baseline due to variations in the back-pressure.

2f. Place the inlet line for the pump in the bottle containing the eluent and place the autosampler wash line into a bottle containing the washing solvent. For best results, the mobile phase and the wash solution should be from the same lot.

2g. Start the pump.

2h. After you fill the flow path in the pump and the injection valve in the autosampler with the mobile phase, turn on power to the autosampler.

Note: Rotating the valve when no liquid is in the injection valve may damage the valve seal.

The autosampler will go through the initialization sequence. At the end of the initialization, the initial screen corresponding to the relevant mode displays.

2i. Press the **WASH** key. This action washes the needle and fills the flow paths of the syringe and the autosampler. Press the **WASH** key at least three times. During this step, check that the wash liquid flows evenly, and there are no leaks. The waste liquid should flow into the autosampler´s waste bottle.

3. Setting Autosampler Parameters

3a. Figure 5-2 shows the initial screen.

STEP	VIAL	VOL(µl)	INJ	TIME(min)	[S]
1	1	10.0	1/1	0.0	

Figure 5-2 Initial Screen

Choose the sequential mode. When you choose the sequential mode, the initial screen shown in Figure 5-2 appears. If this screen does not show, press the **MODE** key for mode selection.

3b. Refer to Section 2.3.2 for instructions on entering conditions. Press the **UTILITY** key and enter the conditions that are given next. Use the **ENTER** key to cycle through the display and to verify the current settings.

RACK CODE : 1
SYRINGE SPEED : 1
INJ. METHOD NO. : 1 (Cut method)
CUT VOLUME, LEAD VOLUME : 30
REAR VOLUME: 30
RACK PARAM : RACK CODE=1
$X_1 = 19$
$X_2 = 154$
NX = 10
$Y_1 = 0$
$Y_2 = 154$
Ny = 12
Z = 39
P = 1
NEEDLE WASH STROKES : 1
NEEDLE WASH SPEED : 5
INJ. PORT WASH STROKES : 1
INJ. PORT WASH SPEED : 5
SYRINGE VOL CODE : 2

3c. Press **SET PROG** and set the following conditions.
CALIB = 0 (NO)

NO.	VIALS	INJ.VOL.	INJ/VIAL	STOP TIME
1	1-1	10	10.0	0

Press **ESCAPE (END)** to complete the setting of conditions.

3d. Set the pump flow rate to 1.0 ml/min.

3e. Set the detector wavelength to 254 nm.

3f. Integrator parameter conditions

SENS : 10
SAMP PERIOD : 100
N-METHOD : 1
STOP TIME : 15
ATT : 9 or 10

Note: Under this condition(STOP TIME: 15), the data for ten injections is recorded together as a single chromatogram. The print out is made in the format given in Figure 2 of the SOP in Section A.3. You need making the change in the D-line connection given in the step 2b.
To obtain the reports for each injection, set the STOP TIME to 2 min. You can use the usual D-line connection in this case.

4. Operation

4a. Wait for stabilization of the pump, column, etc. Wait until the baseline is stable.

4b. Press the **WASH** key. This action washes the needle and the injection port and fills the syringe flow path with washing liquid.

4c. Place a vial containing at least 1 ml of the test sample in rack position number 1 and place the rack in the autosampler.

Notes:
1. Use a clean and dry sample vial.
2. Ensure that the sample vial contains at least 1 ml of sample. If you use a smaller sample, air may aspirate into the system.
3. Orient the sample vial septum so that the milky white surface is on the top.

4d. Press the **START** key to start operation of the autosampler. Press the **START** key to start operation of the integrator at the same time.
The autosampler will carry out the analysis 10 times. For maximum accuracy, ensure that the temperature of the laboratory does not vary significantly. While the run is taking place, the operation conditions appear on the display.

4e. Upon completion of the analysis, "END" displays and operation of the system stops. After an additional 15 minutes, "ALL END" displays

4f. To initialize the system for the next run, press any key (e.g., **CL**). Pressing any key erases the **END** or **ALL END** notation on the display and the initial screen appears.

4g. To analyze additional samples, place the sample(s) in the rack and press **START**.

4h. At the conclusion of the analysis, flush the system to remove buffers, salts or potentially corrosive solvents. Turn off the power. Empty the waste bottles.

5. Data Check

Determine the coefficient of variation (CV) of the peak area via the following calculations:

Average:

$$\overline{X} = \frac{X_1 + X_2 + \ldots + X_n}{n}$$

Standard deviation:

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n}(X_i - \overline{X})^2}{n-1}}$$

Coefficient of variation:

$$CV = \frac{\sigma}{\overline{X}} \times 100(\%)$$

Note that the observed Coefficient of Variation (CV) depends on the performance of the entire chromatographic system including the autosampler, pump, and detector. As an example, if the baseline is very noisy (perhaps due to a pump or detector problem), the CV may be greater than 0.5% because of the incorrect measurement of peak areas.

Notes:

1. Make certain that all components of the system are stable (warmed up) before determining the CV.
2. If the CV is not satisfactory, check the validity of the baseline employed by the data processor. It may be necessary to redraw the baseline, to recalculate the area and to re-determine the CV.
3. If the temperature is above 30°C, air bubbles may form in the syringe. These bubbles will degrade the reproducibility of the system. The presence and extent of these bubbles is a function of the solubility of oxygen and nitrogen in the solvent you are using.
4. Never add a standard sample into a vial containing residual standard sample, or a concentration gradient will occur to cause poor performance. To avoid this, be sure to put a standard sample into a new, clean vial.

5.3 Positioning the Mechanism

5.3.1 Adjusting the Height of the Vial Detection Lever

The position of the vial detection level is set for sample vials with a height of 35 mm (including the cap thickness). If you use a vial of a different height, adjust the vial detection lever so that the gap between the top face of the vial and the bottom of the vial detection lever is 5 mm when the needle is raised to the uppermost end (home position).

Figure 5-3 shows the detection lever adjustment. To adjust the lever, loosen the screw and adjust the lever so that gap between the top face of vial and the bottom of the detection lever is 5 mm when the needle is raised to the uppermost end (home-position). After proper adjustment of the lever, tighten the screw.

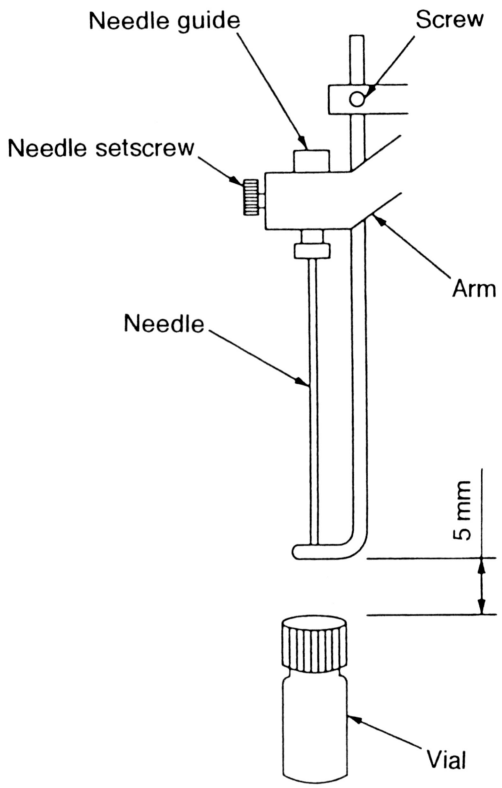


Figure 5-3 Adjusting the Vial Detection Lever

5.3.2 Adjusting the Needle Position (height)

You must adjust the needle height to that of the injection port. Specify the programming mode and enter **TUBE 0 ENTER 1 ENTER** to move the needle to the injection port. Then loosen the needle setscrew, press the needle down gently on the port and secure it. After completing the adjustment, press the **HOME** key to return the needle to its home position.

Note: The factory height adjustment of the needle is for use with the standard sample vials (1.5 ml) and the standard rack (1.5 ml × 120).

Note: If the needle position is improper, liquid may leak from the injection port and the instrument performance may degrade (poor reproducibility, heavy contamination).

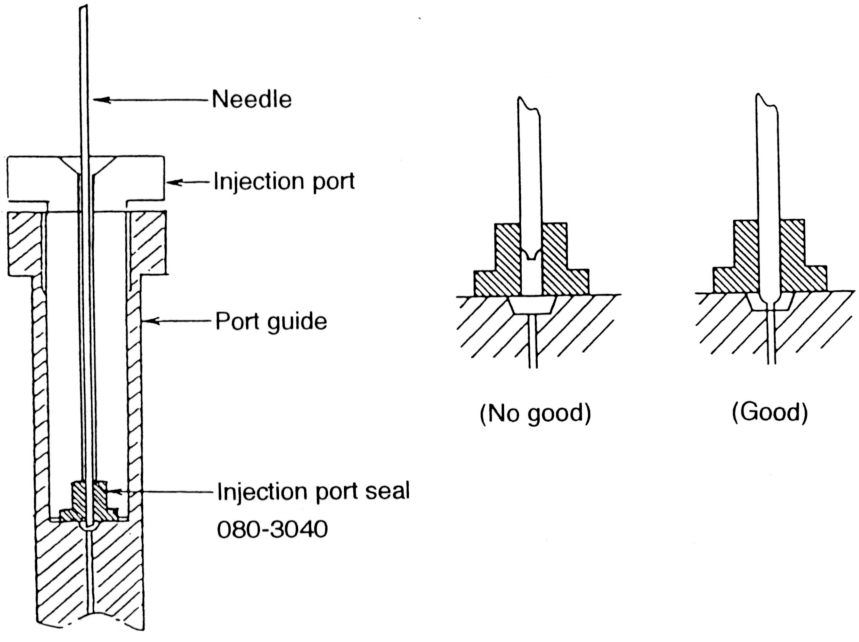


Figure 5-4 Needle Position

5.4 Troubleshooting

5.4.1 Troubleshooting Table

Table 5-2 provides a detailed summary of commonly observed problems, the cause of the problem and a series of suggested solutions.

Table 5-2 Troubleshooting

No.	Symptom	Cause	Remedy
1	The instrument does not operate after turning on power.	a. The fuse is not present.	a. Insert a new fuse (T2AL/250V).
		b. The fuse is blown.	b. Insert a new fuse (T2AL/250V).
2	Error appears on the display.	a. Improper measuring conditions are entered.	a. Perform the action indicated on the display. Clear the error message by pressing any key.
		b. Abnormal operation of the system.	b1. Check if anything is in the path of the mechanism.
			b2. Check if the flow path is clogged or not.
3	ERROR VALVE! displays. (Operation of the injection valve is abnormal.)	a. The injection valve has been overtightened.	a. Adjust it properly. If adjusting it does not stop leakage, replace the injection valve seal.
		b. The injection valve seal is worn.	b. Replace the seal.
			Note: If the injection valve error displays, turn off power. Turning it off clears the error indication. Correct the problem, turn the power on again.

(cont'd)

No.	Symptom	Cause	Remedy
4	Reproducibility of analytical results is poor.	a. Air is trapped in the flow path.	a1. Repeat the WASH operation several times to get rid of air.
			a2. Wash the flow path.
		b. The injection port leaks.	b. Replace the needle seal.
		c. The syringe valve leaks.	c. Replace the valve seal.
		d. The injection valve leaks.	d. Check or retighten the injection valve.
		e. Tubing in the flow path leaks.	e, Retighten or recheck the fittings
		f. The syringe leaks.	f. Clean or replace the syringe.
		g. The sample volume is too small.	g. Increase the sample volume.
		h. The washing liquid bottle is contaminated.	h. Wash the bottle and replace the liquid.
		i. The sample vial is con-terminated.	i. Replace the vial.
		j. The sample has evaporated or degraded.	j. Replace the sample.
		k. The needle is clogged.	k. Clean it.
		l. Excessive variation in the ambient temperature.	l. Move the unit to a location where the temperature variation is smaller.
5	No peak is observed.	a. An insufficient sample volume is used.	a. Increase the sample volume.
		b. The injection port leaks.	b. Replace the needle seal.
		c. The flow path has clogged.	c. Check the piping and/or valve.
		d. The column is faulty.	d. Check the column.
		e. The detector or data pro-cessor is faulty.	e. Check the appropriate unit.
6	"ERROR-D-LINE COMMUNICA-TION" message cannot be cleared.	The system is operating in the D-Line communication mode. During operation, the Integrator (or other master station) was turned off or the cable is disengaged.	Check all connections, then turn off the Integrator and then turn it on again.

5.4.2 On Occurrence of Failure

If a failure is encountered with this instrument, notify your local sales representative or nearest Hitachi service office.
Refer servicing to qualified service personnel who have received relevant technical training.

⚠ WARNING

Beware of Electric Shock!

Potentially Dangerous Voltages are Present within the Instrument.

Before removing the instrument cover for replacement or adjustment of inside parts, be sure to turn OFF the power switch and unplug the power cord.

⚠ CAUTION

Explosion of Lithium Battery!

- This instrument uses a lithium battery for memory backup. It will explode if not handled properly.
- Never recharge, disassemble or incinerate the lithium battery. When discarding the lithium battery, treat it separately from other waste.
- When it becomes necessary to replace the lithium battery with a new one, notify your local Hitachi sales representative or nearest service office.
- Refer servicing of lithium battery replacement to qualified service personnel who have received relevant technical training.
  After the warranty period of this instrument, replacement service is available at charge.

6 PARTS REPLACEMENT

6.1 Consumables and Spare Parts

6.1.1 Consumables

Table 6-1 includes a list of consumable items. The consumption of the sample vials, septa and caps depends on the number of samples you run. Maintain a small stock of each item (2 or 3 port seals, and one of each of the other items) for replacement. Spare consumable items will ensure the maximum uptime of the system.

Table 6-1 Consumable Parts List

Part No.	Name	Location	Yearly Consumption	Remarks
855-2370	1.5 ml sample vial (144 pcs)	Vial	100 to 200 pcs	
855-2351	Septum (for 1.5 ml) (100 pcs)	Vial	500 to 1000 pcs	
855-2371	Pierced cap (for 1.5 ml)(100 pcs)	Vial	100 to 200 pcs	
080-3040	Injection port seal	Injection port	2 to 3 pcs	See 6.2.
080-1390	Injection valve seal	Injection valve	1 pc	See 6.5.
810-3095	Syringe valve seal	Dilutor	1 pc	See 6.6.
810-3097	Dilutor valve stator	Dilutor	1 pc	
080-1064	Syringe (0.5 ml)	Dilutor	1 pc	See 6.4
810-3030	Needle	Flow path	1 pc	See 6.3
635-6247	Needle sleeve	Flow path	1 pc	See 6.3
810-3087	Connection tube (1 mm ID × 1 m)	Flow path	1pc	Tube between syringe and needle

6.1.2 Spare Parts

Table 6-2 lists items that you need to replace from time to time, but are not consumable items.

Table 6-2 Spare Parts List

Part No.	Name	Location	Remarks
655-1395	Filter	Washing liquid aspirating section	See 1.2.2
-	SUS pipe (ϕ 0.5)	For pump connection	Various types for pump connection
-	Setscrew	Injection valve	Various types
J821335	Fuse T2AL/250V		

6.2 Injection Port Seal

Replace the injection port seal after approximately 3000 injections.

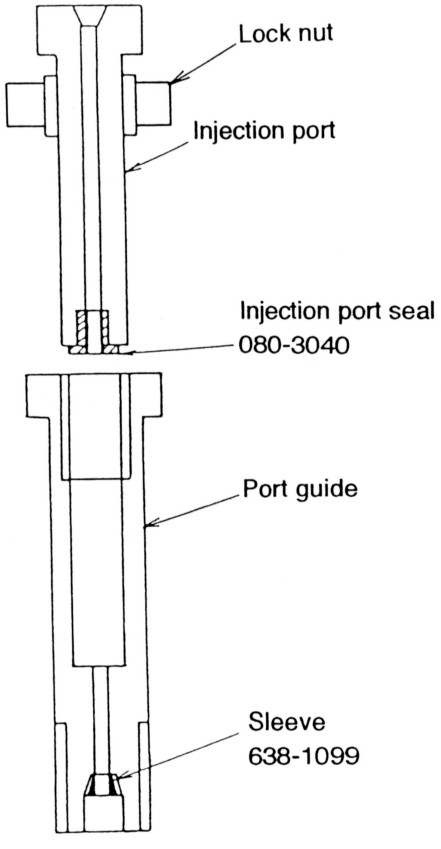


Figure 6-1 Structure of Injection Port

Note: Turn the power off before replacing the injection port.

1. Loosen the lock nut.

2. Remove the injection port and replace the injection port seal.

3. Insert the injection port into the port guide and tighten it securely.

IMPORTANT:

1. After replacing the injection port seal, turn the power on. After the initialization of the system, press the **WASH** key and make certain that there are no leaks at the seal.
2. If the tip of needle is deformed, the useful life of injection port seal may be significantly reduced. Inspect the needle carefully and replace if it is bent or dull.
3. Make certain that the injection port is securely tightened. A loose injection port reduces the performance of the autosampler.

6.3 Needle Replacement

To replace the needle:

1. Loosen the setscrew and remove the needle from the arm.

2. Loosen the setscrew (A) with a wrench.

3. Pull out the needle.

4. Put the setscrew (A) and the needle sleeve on a new needle and insert that assembly into the needle guide as far as it goes. Tighten the setscrew (A) securely. Make sure that there is no gap between the needle end and the needle guide hole.

5. After replacement, see Section 5.3.2 to adjust the needle position.

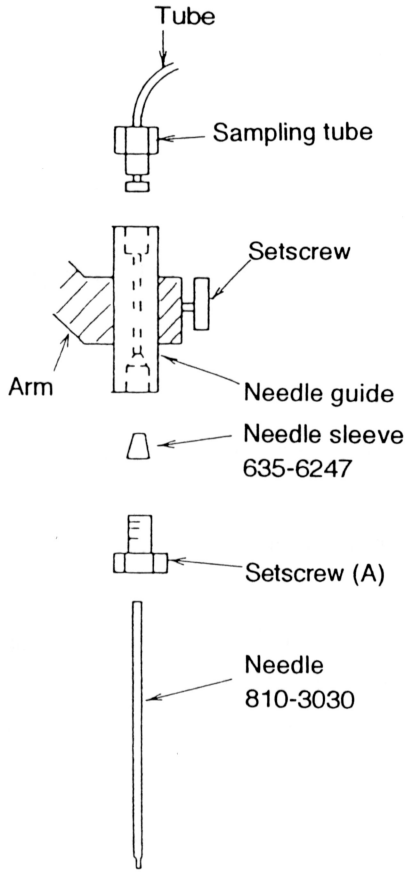


Figure 6-2 Needle Replacement

6.4 Syringe Replacement

1. The thread at the top end of the syringe is such that you can remove it by turning it counterclockwise.

2. Before placing a new syringe in the system, fill it with distilled water (or washing liquid).

3. When mounting the syringe in the system, first position it in the plunger groove. With the plunger correctly positioned in the plunger groove, raise the plunger and tighten the top part (which is threaded).

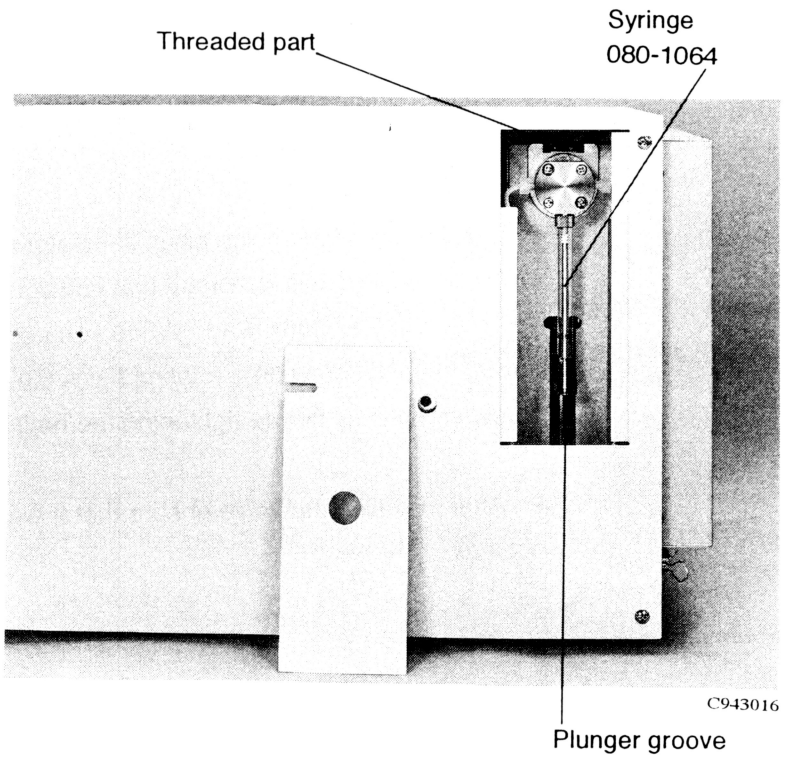


Figure 6-3 Syringe Replacement

IMPORTANT:

1. Do not move the plunger when the syringe interior is dry. Moving the plunger seal when it is dry causes excessive wear because of insufficient lubrication.
2. Be sure to securely tighten the valve screw on the syringe (it should be finger tight). If you do not tighten the seal completely, the joint seal will deteriorate and you will observe poor performance.

6.5 Replacing the Injection Valve Seal

Replace the injection valve seal in the following manner (see Figure 6-4 for the structure of the Injection Valve).

1. Loosen the three stator setscrews using a hexagonal wrench, and remove the stator from its ring.

2. Pull out the injection valve seal.

3. Mount a new injection valve seal so that the notch mark on its circumference aligns with the rotor pin as shown on the bottom of Figure 6-4.

4. Position the rotor so that the rotor pin turns between the stop pins.

5. Engage the stator guide pin in the stator hole and mount the stator on its ring. It is important that you tighten the three stator setscrews in a uniform way. When you tighten the screws, apply torque to each screw on a rotating basis (i.e. do not tighten one screw completely before tightening the next screw).

6. After installing the valve ensure that the injection valve does not leak.

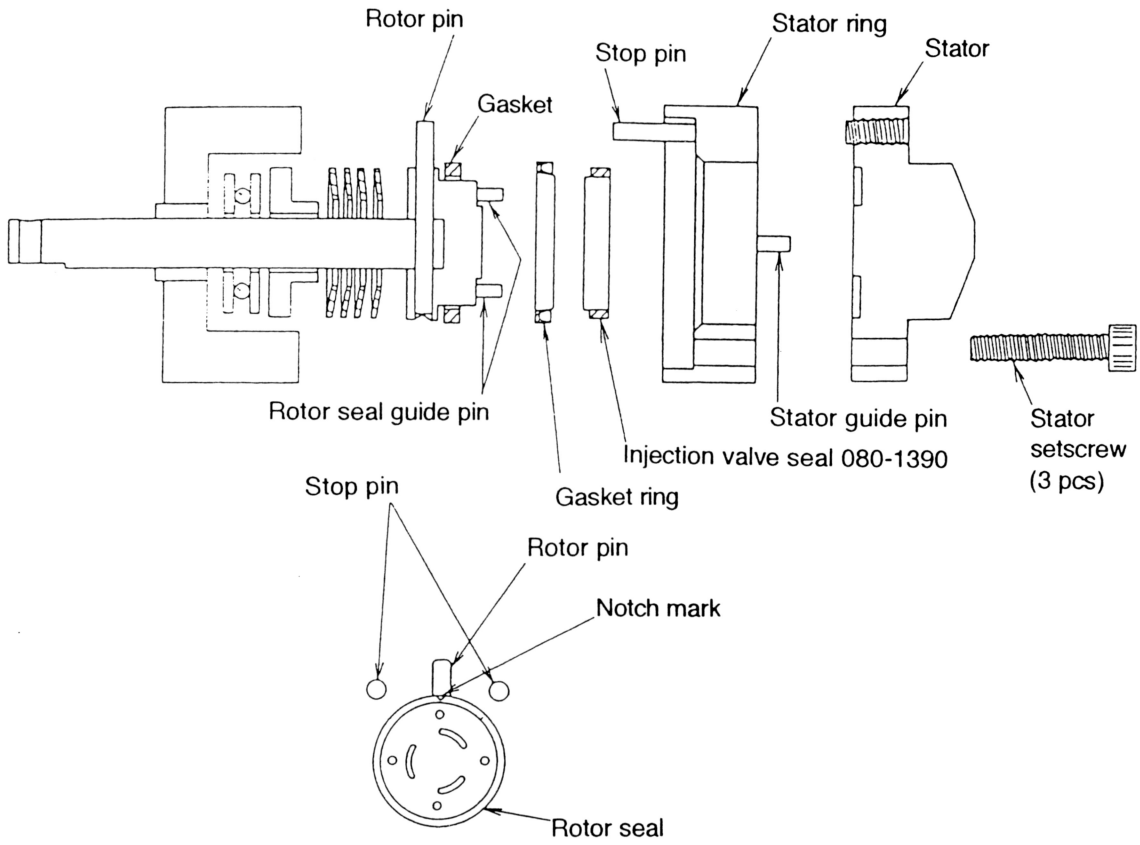


Figure 6-4 Structure of Injection Valve

6.6 Replacing the Syringe Valve Seal

Replace the syringe valve seal in the following manner:

1. Disconnect the tubing from the washing liquid bottle.

2. Remove the syringe.

3. Loosen the two syringe valve setscrews and remove the valve (Figure 6-5).

4. Loosen the stator setscrews and remove the stator.

5. Pull out the syringe valve seal.

6. Mount a new syringe valve seal (Figure 6-6).

7. Mount the stator.

8. Mount the syringe on the stator. The end of the shaft is slanted to indicate the proper orientation of the shaft. In addition, note the orientation of the rotor groove and the pinhole shown in Figure 6-6.

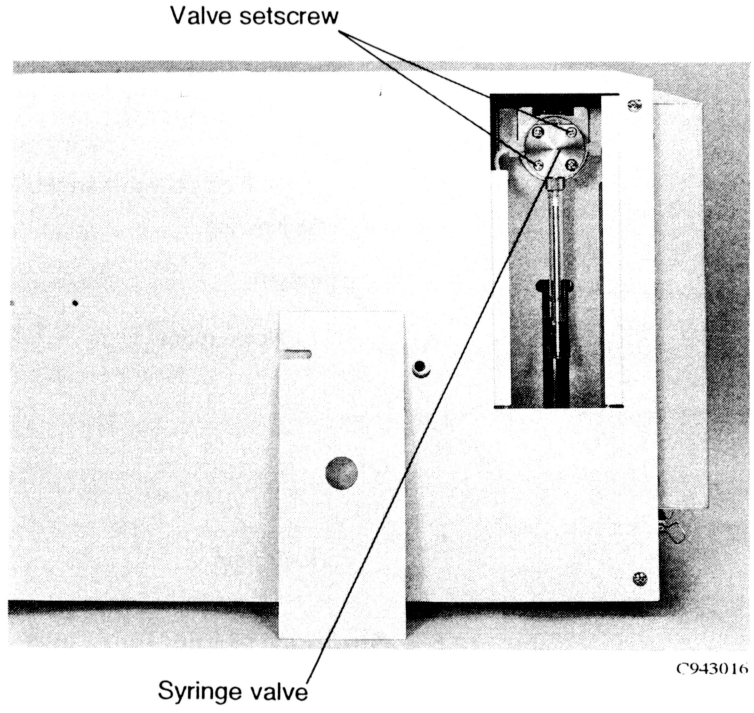
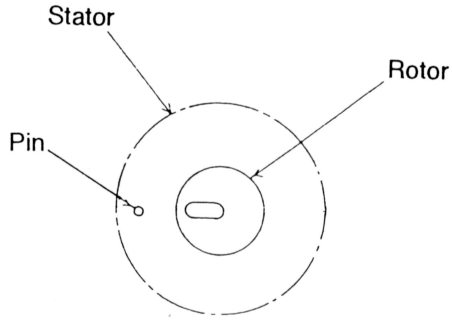


Figure 6-5 Dilutor Valve



Mount so that the rotor groove will position at the pin hole.

Figure 6-6 Orientation of Rotor and Stator

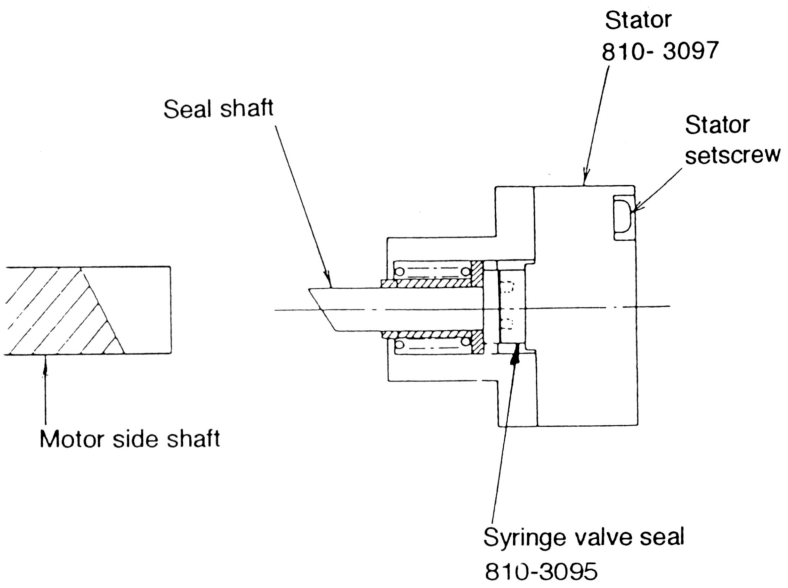


Figure 6-7 Structure of Syringe Valve

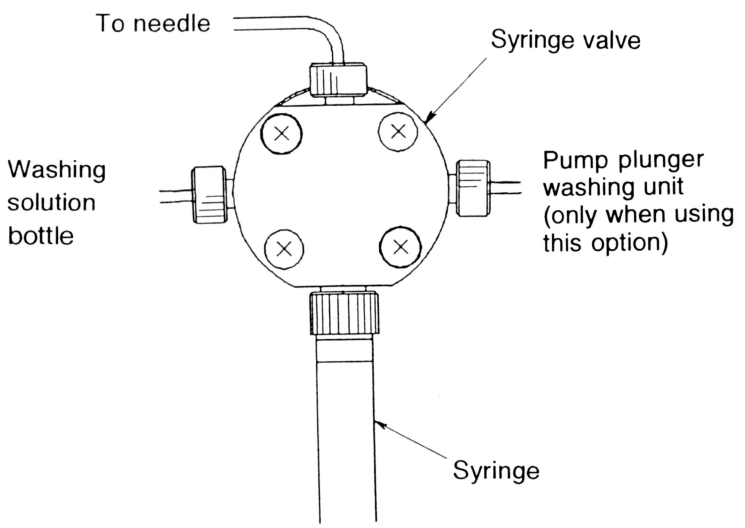


Figure 6-8 Tube - connection of Syringe Valve

APPENDIX

A.1 Error Message List

No.	Error Message	Description
1	ERROR-X!	Arm X (transversely) movement error
2	ERROR-Y!	Arm Y (longitudinally) movement error
3	ERROR-Z!	Needle Z (vertically) movement error
4	NO TUBE!	Test tube (vial) detect error
5	ERROR-SYRINGE (1)!	Syringe's movement error
6	ERROR-SVALVE (1)!	Syringe valve's movement error
7	ERROR-IVALVE!	Injection valve's movement error
8	ERROR-ACC!	Accessory movement error
9	ERROR-PARAMETER!	Parameter is out of range
10	ERROR-D-LINE COMMUNICATION!	Unsuccessful D-line communication with the master station. (Cause: Power switch of master station (data processor) has been turned off or the D-line cable is not connected.) (Remedy: Turn on the master station and/or connect the cable.)
11	ERROR-NO PROGRAM!	No program is present in the editor buffer
12	ERROR-NO FILE	The specified program number file cannot be found in the RAM disk when you process the **FILE** functions of LOAD, DELETE and DIRECTORY.
13	ERROR-SYNTAX!	1) The DO and LOOP numbers do not matched. 2) DO and LOOP sequence is reversed.
14	ERROR-OUT OF MEMORY(1)	No area is available to add a command during the TEACH or the EDIT processing.
15	ERROR-OUT OF MEMORY(2)	During the SAVE operation, the program in the editor buffer can not find a continuous area in the RAM DISK. (Countermeasure: Delete unnecessary files. Condense to form one integrated area of all the separated free areas.)
16	ERROR-COMMAND!	An illegal command was found in the program. (Countermeasure: Press the **TEACH** key, select 1) YES to delete the program in the buffer area. If the program was saved in the RAM DISK, delete it with the DELETE function.)

(cont'd)

No.	Error Message	Description
17	ERROR-RS-232C	Data or and @ mark from an external computer could not be transferred to the autosampler within the 60 second time limit. (RS-232C mode)
18	CHECK VIAL NO.	1) The vial No. exceeds Nx•Ny (total number of vials) of the RACK parameter was set when the analysis started (when the START was pressed). 2) The starting vial No. is greater than the last vial No..
19	CHECK TOTAL VOL.	1) The CUT VOL. or INJ VOL. is greater than the sum of the CUT VOL. + INJ VOL. + 55 µl for the current syringe capacity. 2) The 'FEED VOL' or 'INJ VOL' parameter value is larger than the actual capacity of the current syringe (feed volume + injection volume + 55 µl). 3) The 'WASTE VOL' or 'INJ VOL' parameter value is larger than the actual capacity of the current syringe (waste volume + injection volume + 55 µl).
20	CHECK SYRINGE SPEED!	The syringe speed set with the UTILITY function (SYRINGE SPEED, WASH SPEED) exceeds the upper limit values given in Table 2-4 (when a 2.5 ml or a 5 ml syringe is used).
21	CHECK START STEP NO!	1) When starting an analysis (**START** key pressed), START STEP NO. not present in the program. 2) When setting the START STEP NO. with the UTILITY function, the system is trying to access a step is not present in the program.
22	CHECK D-LINE MODE!	The D-line communication mode is not set up or the PRINT function (UTILITY menu) is trying to print but the printer is not active.
23	SYSTEM BUSY:CHECK OTHER MODULE STATUS	Analysis cannot start because the other module connected is busy.
24	ERROR-COOL UNIT1	Error of the thermoelectronic rack (No.1)
25	ERROR-COOL UNIT2	Error of the thermoelectronic rack (No.2)
26	ERROR OPVALVE	Error in the optional valve

A.2 Connecting Components via the D-line Communication Feature

1. Outline The L-7000 series system uses two types of contact signals.

1a. Contact Signals (START, STOP, BUSY) Entering the D-line Connector These are contact signals having input/output functions which are common to each unit. These signals come from the START(IN/OUT), STOP(IN/OUT) and BUSY(IN/OUT) lines and are valid only when you turn off the D-line (via the setup function of each unit.)

1b. Individual Contact Signals of Each Unit These are contact signals which are input and output via a 3-pin connector, and their names and functions vary with each unit.
The name indicates the function of each connector.

Example of output contact signal name: EVENT of pump
Example of input contact signal name : LAMP OFF IN of detector
SERIES START IN of autosampler

2. Contact Signals Entering the D-line Connector Each unit has two D-line connectors (except for the data processor which has one) at the rear panel. When you connect the components in series via the D-line cable as shown in Figure A-1, you send a signal from one unit to any other unit in the system. This signal transfer automates the operation of the entire system.

Use the cables listed next to automate operation via contact signals with the L-6000 series and/or D-2000/2500 or D-6000/6500.

2a. For Achieving only an Automated Start

Part No.	Part Name	Opposing Terminal	Opposing Device (Example)
810-7634 (810-7514	SDIO (START 3P) cable FDIO (3P) cable)	3P connector	L-6200, D-6000
810-7633 (810-7513	SDIO (START M3) cable FDIO (M3) cable)	M3 terminal	L-5000
810-7632 (810-7512	SDIO (START M4) cable FDIO (M4) cable)	M4 terminal	D-2500/ 2000

2b. For Achieving an Automated Ready/Busy and Stop as well as Start

Part No.	Part Name	Opposing Terminal	Opposing Device (Example)
810-7631	SDIO cable	3P connector	L-6200,
(810-7511	FDIO cable)		D-6000

If the opposing terminal is other than a 3P connector, connect a separate cable to the end of the SDIO cable. ( ): For connection with the instrument bearing the CE conformity marking.

Notes:

1. Use the Hitachi cables for the transmission of the contact signals.
2. Use the relay box (P/N 810-7630) for automating via contact signals the operation of a system that includes two (or more) non-Series 7000 components.
3. Observe that the contact closures are polarized (i.e. the two terminals are not equivalent). When connecting components, use care to maintain the correct polarity.
4. Apply only a maximum of 30 V across the contact terminals.

5. Observe that when you short a contact terminal (i.e. a signal is sent from one component to another component), a maximum current of 10 mA will flow. If you connect multiple units, this current will be additive. Contact circuits should be able to withstand a current of 0.1 A.
6. Note that the maximum current of the start signal contact output is 0.1 A. Check the maximum current of the contact signal of the reception circuit before making a connection.
7. See Figure A-2 for a diagram of the D-line contact circuit.

3. Type of Contacts on a Component

3a. Contact Input Terminals These are contact signal input terminals for control using the contact signals from an external switch, relay, etc. Activate the signal by shorting the terminal for 0.1 second or longer. Figure A-3 shows the input circuit.

3b. Contact Signal Output Terminals These are contact signal output terminals for control using the contact signals of an external unit. Figure A-4 shows the output circuit.
The contacts can carry up to 0.1 A at 30 V. Make sure the load does not exceed this rating.

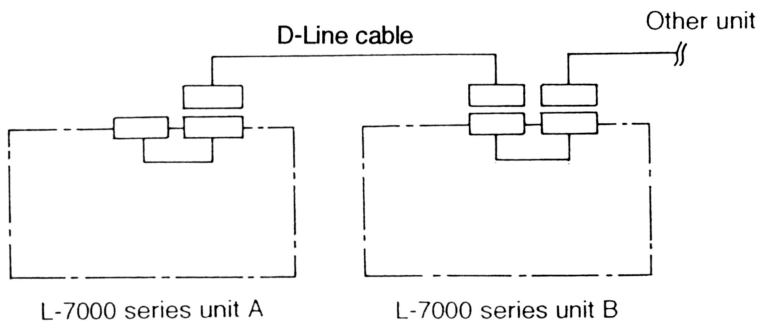
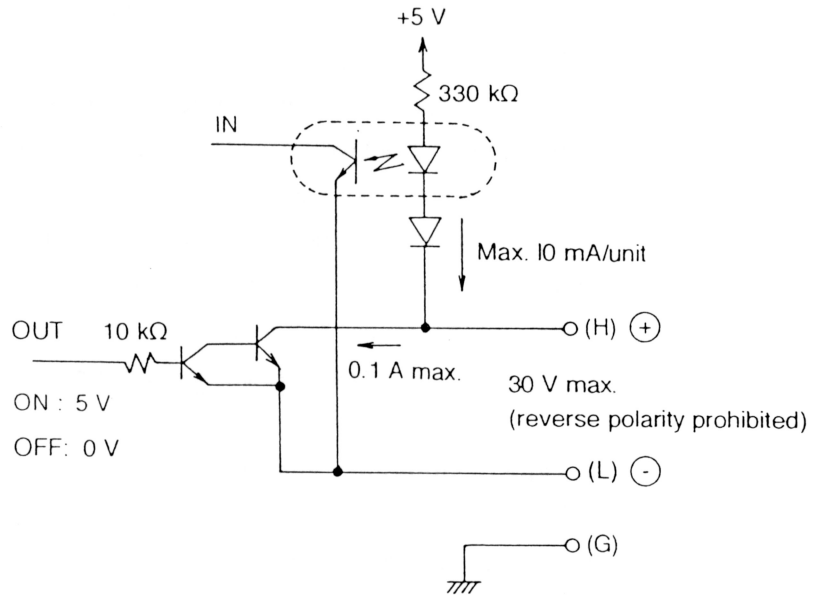
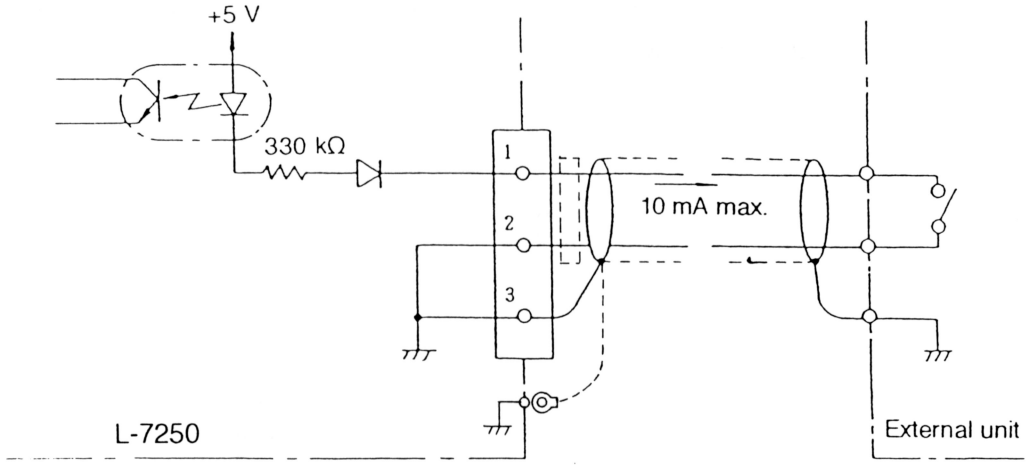


Figure A-1 D-Line Cable Connection



---: For connection with the instrument bearing the CE conformity marking.

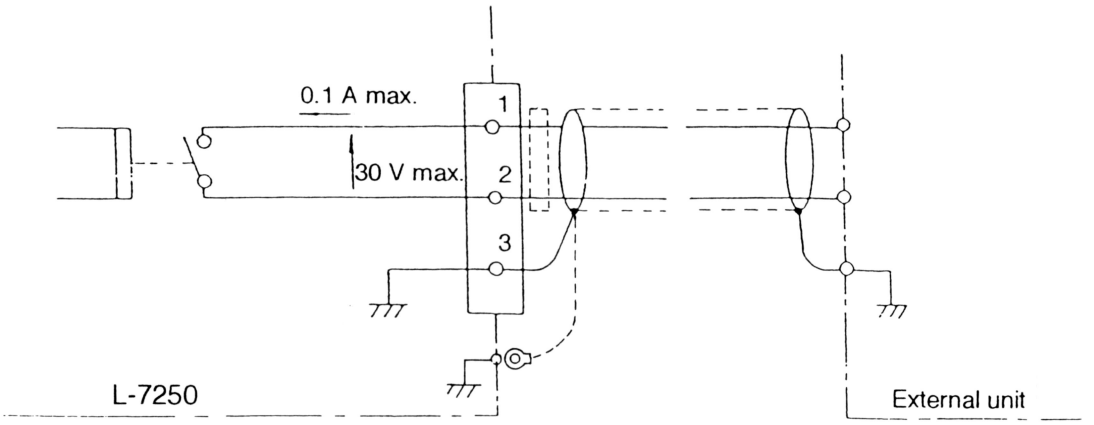
Figure A-2 D-line Contact Circuit Configuration



Activated upon shorting contacts of external unit.

---: For connection with the instrument bearing the CE conformity marking.

Figure A-3 Contact Signal Input Circuit



---: For connection with the instrument bearing the CE conformity marking.

Figure A-4 Contact Signal Output Circuit

A.3 Terminology

All volume injection method : A kind of sample injection method.

ASPIRATE (ASP) : Liquid aspiration command.

Calibration : Operation in which a calibration curve is prepared for calculating a concentration of sample.

Cut injection method : A kind of sample injection method.

D-Line : Digirtal network dedicated for analytical system.

Deaeration(Degassing) : Removal of air (gas) from wash solution.

Error message : To be displayed upon occurrence of an error or fault in the instrument.

Initial screen : After power-on, the initial screen appears upon completion of initialization.

Initialize : The instrument is initialized.

Injection : A sample is injected.

Injection port : Used for introducing a sample into the injection valve.

Injection valve : Used for sample injection changeover to the high-pressure part.

Local : Indicates the internal control status.

OUT : Contact signal output command.

Pattern code : Indicates an analytical sequence of samples on the rack.

Quick sample : For a quick sample, analytical operation for a series of samples can be interrupted in emergency.

Rack : Samples are set on the rack for analysis.

Remote : Indicates the external control status.

Septum : A seat that covers a sample vial.

START : Analytical operation for a series of samples is started.

Stop time : A period of time is required to attain a chromatogram in analysis of a sample. When this period of time elapses, analytical operation is resumed for the next sample.

Syringe : Equipped to aspirate/discharge liquid in combination with the plunger.

Table setting screen : Used for setting up analytical conditions for each sample.

Utility : Used for setting up operating conditions of the instrument.

Warming-up : The instrument is warmed up. Sufficient warming-up is necessary to ensure stable analysis.

Washing port : Used for washing the needle.

INDEX

A

Adjusting the needle position 5-11
ALL END 1-11, 2-34, 5-9
All volume injection method 2-36, 3-2
Ambient humidity 3-7
Ambient temperature 3-7
Analysis table 2-7, 2-9
Analysis time 2-8, 3-6
Analytical operation A-8
APL PARAM 2-20, 2-22
Arm 1-2, A-1
ASP (Aspirate) A-2, A-8
Assembly 4-4
Autosampler 1-1, 2-1, 2-5
Average 5-9

B

Bench 4-2
BUSY A-3

C

Cable connections A-5
Calibration 2-7, A-8
Cap 1-7, 6-1
Capacity 4-2
Checking the performance and specifications 5-3
Checking the reproducibility 5-3
Cleaning 5-3
CLEAR 1-5
Coefficient of variation 5-9, 5-10
Column 1-1, 1-8, 4-8
Command A-1, A-8
Connection tube 6-1
Consumables 6-1
Contact input A-5
Contact output A-5
Contact signal 4-14, A-3
Contents 4-1

Cover 4-6
Cut injection method 2-5, 3-2
Cut volume 1-12, 2-14, 2-35, 3-3

D

D-line A-1
Data check A-1
DELETE A-1
Detector 1-1, 1-11, 5-5, 5-10
Detergent 5-2
Dimensions 3-7
DIRECTORY A-1
Display (panel) 1-3, 3-6
DO A-1

E

EDIT A-1
Eluent 1-2
END 1-5, 1-11, 2-34, 5-7
ENTER 1-5, 2-29, 5-7
Error A-1, A-2
Error message A-8
ESCAPE 1-5, 2-30, 5-7
Extran 5-2

F

Feed volume 2-14, 2-15, A-2
FILE A-1
Filter 5-3, 6-2
Flow path 1-3
Frequency 4-2
Fuse 5-13

G

Ground 4-10

I

Initial screen 1-11, 2-14, A-8
Initial setting 2-3
INJ. METHOD 2-13, 2-14
INJ. PORT WASH STROKES 2-22
INJ. VOL 2-9
INJ/VIAL 2-9
Injection port 1-4, 4-8
Injection port seal 6-1, 6-2
Injection valve 1-3, 1-4, 4-8
Injection valve (rotor) seal 6-1
Injection volume 2-8
Injection volume reproducibility 3-6
Input circuit A-6
Installation 4-1
Integrator 1-1, 1-11, 4-12, 5-4, 5-8
Items to be provided by user 4-4

K

Keypad 1-3, 1-4

L

Layout 4-4
Liquid chromatograph 1-1
LOAD A-1
Load resistance coil 5-4
LOCAL A-8
LOOP A-1

M

MODE 1-5, 1-11, 5-6, A-2

N

Needle 1-3, 1-4, 6-1
NEEDLE DOWN SPEED 2-22
Needle height 1-12
Needle replacement 6-3
Needle sleeve 6-1

NEEDLE WASH STROKES 2-22
Number of injections 2-8
Number of injections per sample 3-6
Number of standard samples 3-6

O

Operation flow 2-4
Operation keys 1-4
Operation precautions 2-35
OPTION 2-24
OUT A-3, A-8
Outline of system operations 2-3
Output circuit A-5, A-7
Output terminal A-5

P

Parameter list printout 2-27
Parts replacement 6-1
Pattern code 2-16, A-8
Periodic checking 5-1
Periodic maintenance 5-1
Power consumption 3-7
Power cord 4-10
Power switch 1-3
Pressure resistance 3-6
Principles 3-1
PRINT 2-24
Programming mode 1-5, 2-5
Pump 1-1, 1-2, 5-2, 5-3, 5-4, 5-5

Q

QUICK sample 1-5, 2-4, 2-29

R

RACK CODE 2-13
Rack 1-3, 3-6, A-1, A-8
RACK PARAM 2-13, 2-16, 2-18
RACK SELECT 2-13

RAM A-1
REAR VOLUME 2-14
REMOTE A-8
Replacement of injection valve (rotor) seal 6-6
Rinsing liquid 5-2
Routine operation 1-10, 2-5
RS-232C A-2

S

Sample injection volume 3-6
Sample vial 1-4, 1-7, 4-4, 6-1, A-8
SAVE A-1
Septum 1-7, 6-1, A-8
SEQ 2-12, 2-14
Sequential mode 1-5, 2-3
SET PROGRAM 1-5, 2-6, 5-7
Setting 1 analysis conditions 1-11, 2-6
Setting the rack parameters in the robotics mode 2-20
Signal line 4-12
Solvent 5-2
Space requirements 4-1
Spare parts 6-1, 6-2
Specification 3-6
Standard deviation 5-9
Standard sample 1-12, 5-4
Standard solution 2-7, 2-10
START 1-5, 5-9, A-2, A-8
START STEP 2-12
Step number 2-8
STOP 1-5, 5-9, A-2, A-8
Stop procedure 2-34
STOP TIME 2-8
SVALVE 1-4, A-1
SYRINGE 1-4, A-1
SYRINGE SPEED 2-13, 2-23, A-2
SYRINGE VOL. CODE 2-24
Syringe 1-2, 1-3, 1-4, 6-1, A-1, A-8
Syringe capacity 3-6
Syringe replacement 6-5

T

TEACH A-1
Thermoelectronic rack A-2
Troubleshooting 5-13
Tubing connections 4-8

U

UTILITY 2-6, 5-7, A-2, A-8
Unknown sample 2-10
Unpacking 4-1

V

Valve 1-3, 1-4
Vial detection lever 5-10, 5-11
Vial number 2-8
Voltage 4-1

W

Warming-up A-8
WASH 1-5, 5-6, A-2
Wash port 1-4
Wash solution 1-1, 1-4, 1-8, 2-37
Washing 5-1
Waste solution 1-4, 1-10, 4-9
Waste solution/liquid bottle 1-1, 1-6, 4-5, 5-5
Waste solution/liquid tube 1-6, 4-9
Weight 3-7
Wetted-part materials 3-6
Wiring 4-10

STANDARD OPERATING PROCEDURE (SOP)

This section provides the standard operating procedure (SOP) that tests the performance of the Model L-7250 autosampler. Use this SOP for quality control in GLP situations.

		SOP - | | |	Page 1 of 11

Standard - Operating Procedure SOP

Title:	Testing the function and specifications of the L-7250 Autosampler
Valid since:	1.6.1994
This SOP replaces the version of:	New version, issue 1
Policy:	Establishing a reliable function of the instruments
Responsibility:	Head of Laboratory

	Date/signature	Copy received by:
Written:	.....	Testing Facility Manager Laboratory Manager Laboratory personnel QA department
Reviewed:	.....	
Approved:	.....	

Total number of pages:	11 and Test Report pages
References:	L-7250 Autosampler Operating Instructions

Contents

1. Introduction
2. Testing
3. Documentation: "Test Report"
4. Archiving

1. Introduction

The reproducibility of the sample volume is of critical importance for the precision of HPLC analytical results. Check the reproducibility of the autosampler injection at regular intervals.

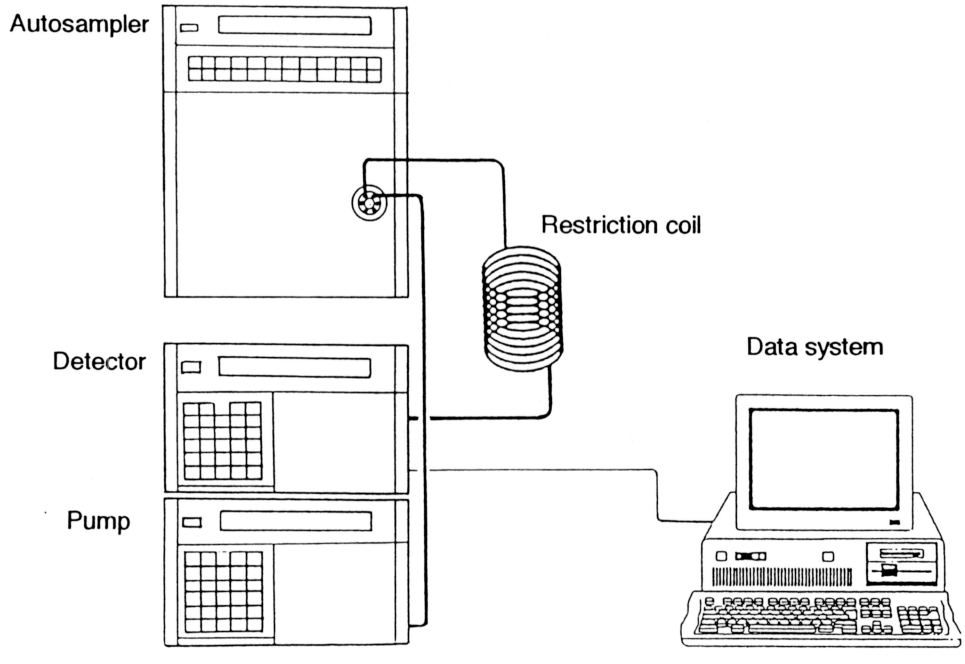


Figure 1 Set up of the HPLC-instrument for the Test

2. Testing

2.1 Test procedure

To determine the injection reproducibility of the L-7250 that is part of an HPLC system, make a series of 10 identical injection volumes using a 40 ppm Perylene test solution.

If you establish that the instrument does not meet specifications, designate the instrument as defective and do not use it until it is serviced or the problem is resolved.

2.2 Testing intervals

Perform the reproducibility check once a month. However, if you observe problems or questionable results, carry out this test right away.

2.3 Apparatus

Use only HPLC-quality (HPLC-Grade) solvents and reagents for the reproducibility test. Employ instrumentation and components of high quality to ensure that they are suitable for analytical experiments.

As shown in figure 1, the HPLC system comprises the following components:

An HPLC pump (e.g. the L-7100 or equivalent instrument) capable of delivering a pulse-free flow of 1 ml/min.

The L-7250 Autosampler to be tested.

A Teflon capillary (10 m long, ID = 0.25 mm) serving as a restriction capillary and simulating the HPLC column.

A UV detector (e.g. the L-7400 or equivalent instrument). Set the measuring wavelength to 254 nm.

A data evaluation system (e.g. D-7500 or a PC with the appropriate chromatography software for the detection and evaluation of the detector signal - e.g. the D-7000 HPLC Manager")

The usual accessories for HPLC, e.g. solvents, waste bottle, HPLC fittings, connecting capillaries (see procedure for details).

2.4 Reagents and test solutions

Degas all solutions prior to using them for this particular test procedure.

The following items should be available:

Methanol (HPLC-Grade) as a mobile phase, wash solution, and for preparing test solutions. 1 ml of the Perylene test solution:

Weigh 8 mg Perylene into a 10 ml volumetric flask. Use an ultrasound device to mix and dissolve the Perylene in 5 ml tetrahydrofuran, THF. Using 195 ml methanol, transfer the solution into a 250 ml brown glass bottle and mix it. The final concentration of the solution should be 40 ppm (40 mg/L). Store the mixture in a refrigerator.

Caution: Perylene is both mutagenic and carcinogenic! Use appropriate safety measures (gloves, fume cupboard). Do not inhale the Perylene powder.

You can order this solution from HITACHI (P/N 810-3105).

2.5 Test procedure

2.5.1 Preparation

When you use the D-7500 Integrator for the data evaluation, connect it to the Autosampler with the start D-line cable (see operating instructions for L-7250 Autosampler and D-7500 Integrator). You can document the individual injections. Upon completion of the injection series, mean values and standard deviations calculate automatically. You may also use the D-7000 HPLC-Manager software (see the software instructions).

Figure 1 shows the HPLC module connections necessary for the test.

Before switching on the system, check that all tubing and electrical connections are correct.

Ensure that the waste tube seats properly inside the waste bottle. Place the waste bottle at a lower level than the L-7250 so that waste solution can flow properly. In order to prevent back pressure, the waste tube from the detector should be inserted directly into the waste bottle. Do not connect the detector waste tube to the L-7250 intermediary waste bottle (see operating instructions for the L-7250).

Fill two bottles with HPLC-grade degassed methanol. Connect one bottle to the aspiration tube (inlet tube) of the pump (mobile phase) and use the other bottle for the washing solution of the L-7250.

2.5.2 Starting the system:

Start the pump following that unit operating instructions. Pump the mobile phase through the system until it is free of bubbles.

When the mobile phase is free of air and flowing through the injection valve, start the Autosampler. Do not start the autosampler if there is no liquid in the injection valve. Damage may occur to the valve seals if the valve is dry.

When you power on the Autosampler, initialization of the unit occurs automatically. The relevant basic data that appears on the LCD display will reflect the active operating mode.

Press the **WASH** key and wait until the washing procedure ends.

Repeat the washing procedure three times.

Ensure that there are no leaks and that the pump is operating smoothly (check the operating pressure and the flow rate).

2.5.3 Entering the test conditions:

Switch the Autosampler to SEQUENTIAL ANALYSIS (if necessary, refer to the operating instructions). You may have to press the **MODE** key to observe the following display.

```
STEP  VIAL  VOL µl  INJ  TIME (min)        (S)
 1    1-1     10    1/1     0.0
```

Press the **UTILITY** key and enter the following conditions:

RACK CODE	:	1
SYRINGE SPEED	:	2
INJ. METHOD NO.	:	1
CUT VOLUME	:	30
LEAD VOLUME	:	30
REAR VOLUME	:	30

(continued on next page)

RACK PARAMETER (for the standard rack):

:RACK CODE = 1
:X1 = 19 Y1 = 0
:X2 = 154 Y2 = 154
:Z1 = 39
:Nx = 10
:Ny = 12
: P =1

NEEDLE WASH STROKES : 1
NEEDLE WASH SPEED : 5
INJ. PORT WASH STROKES : 1
INJ. PORT WASH SPEED : 5
SYRINGE VOL CODE : 2

Press the **SET PROG** key and enter the following conditions:

CALIB = 0

STEP	VIALS	INJ VOL	INJ/VIAL	STOPTIME
1	1-1	10	10	0.0

Set the pump flow rate to 1 ml/min and the detector wavelength to 254 nm.

Wait until the pump, column and detector reach equilibrium and until you observe a steady baseline. Set the attenuation of the integrator or the scale expansion of the Y-axis of the data system to produce a clearly visible signal (ca. 1 - 5% of scale). You will then be able to clearly observe the baseline noise. Allow at least a 30 minute equilibration time.

Press the **WASH** key and wait until the washing procedure ends. Repeat this washing process at least three times.

Place a sample vial containing 1 ml of the standard test solution in rack position 1 and place the rack in the Autosampler.

Press the **START** key. The autosampler will make 10 sample injections automatically and in sequence. You can follow the analysis procedure on the autosampler display or on the PC monitor if one is being used

If you record the peaks with a recorder or an integrator that does not permit re-integration of the peaks on a full-format basis, set the attenuation so that you can see the apex of the peaks in the trace. If necessary, repeat the series of measurement .

On completion of the injection series, **END** will display on the L-7250 Autosampler.

To return the system to the run state, press the **CL** key.

2.6 Evaluation

At the completion of the procedure, you should observe 10 HPLC peaks as shown in Figure 2. Evaluation takes place by area integration on the part of the integrator or by the PC integration software. In either case, the system uses the following mathematical functions.

Mean value :

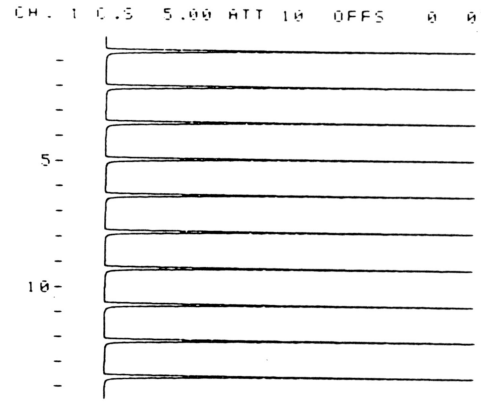
$$\overline{X} = \frac{X_1 + X_2 + \ldots + X_n}{n}$$

Standard deviation :

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n}(X_i - \overline{X})^2}{n-1}}$$

Coefficient of variation :

$$C_v = \frac{\sigma}{\overline{X}} \times 100(\%)$$



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METHOD: L-7200------ TAG: 4 CH: 1

FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	HEIGHT	AREA	CONC	BC
1	0.57	458714	2213793	10.011	BB
2	2.02	457826	2211033	9.999	BB
3	3.46	458503	2212514	10.005	BB
4	4.91	457189	2208649	9.988	BB
5	6.35	457982	2214344	10.013	BB
6	7.79	457164	2210421	9.996	BB
7	9.23	456058	2201848	9.957	BB
8	10.68	456623	2205414	9.973	BB
9	12.12	459359	2222261	10.049	BB
10	13.57	457792	2213324	10.009	BB
TOTAL		4577210	22113601	100.000	
PEAK REJ :		0			

Figure 2 Evaluating the Test Results

3. Documentation: "Test Report"

The Autosampler is within manufacturer's specifications if the coefficient of variation is equal to or less than 0.5%.

If the instrument does not meet specifications, label the instrument as defective and do not use until the problem is corrected or service is performed.

The "Test Report" result given next and the evaluation form are examples of this SOP. Use a similar form to document the results you achieve. The Test Report should include:

Serial number of the Autosampler (S/N)
Test date
Date of next test
Name of the person conducting the test
The final results obtained

For documentation, you should report the results and the data. Record them in the laboratory log book in chronological order. Include the original chromatogram with the 10 peaks and all the calculations.

Include the corresponding Integrator or PC printout to keep records of the method measuring parameters.

4. Archiving

4.1 Archiving at manufacturer's location

Operating instructions, service documentation and spare parts lists should be available for reference. The manufacturer guarantees that these documents will be available for 30 years.

4.2 User's archiving obligation

The user will keep all documentation, specification, reports, and maintenance tests carried out in connection with GLP testing.

		Test Report	Page 1 of 1

Module	**Autosampler**
Model	**L-7250**
Instrument number	

Specification test

No.	Item tested	Setting	Specification	Result	Tester
1	Reproducibility	Perform 10 Injections of the same amount of a test solution within one series. Evaluate the peak areas	Coefficient of variation less or equal 0.5% For calculation cf. page 7 of this SOP		

Test on: .....

Next test scheduled for: .....

Tested by: .....